MOUNT SINAI
GUSTAVE L.
AND
JANET W.
LEVY
LIBRARY

MOUNT SINAI
GUSTAVE L.
AND
JANET W.
LEVY
LIBRARY

Digitized by the Internet Archive
in 2015

https://archive.org/details/mountsinaijourna5919moun

THE MOUNT SINAI JOURNAL OF MEDICINE

VOLUME 59 NUMBER 1 JANUARY 1992

CONTENTS

FROM BENCH TO BEDSIDE AND BACK

Lactic Acidosis, Ketoacidosis, and Energy Turnover: "Figure" You Made the Correct Diagnosis Only When You Have "Counted" on It—Quantitative Analysis based on Principles of Metabolism *Mitchell L. Halperin, Kamel S. Kamel, and Surinder Cheema-Dhadli* 1

GRAND ROUNDS

Why Kidneys Fail *Lance D. Dworkin* 13

GENERAL ARTICLES

Environmental Hazards and Public Health: Lessons for the Practice of Medicine and for Public Policy *Richard P. Wedeen and Helen E. Sheehan* 23

Diminished Ratio of Estrogen Receptors to Progesterone Receptors in Breast Carcinomas of Women Who Have Had Multiple Miscarriages *Steven Lehrer, Eva Levine, Penny Savoretti, Joan Cropley, Jack Dalton, HK Song, Swan N. Thung, Jack Rabinowitz, and Lynda Mandell* 28

The Correlation between Lupus Anticoagulant and Autoantibodies *Norbert Gleicher, Lisa Harlow, and Albert El-Roeiy* 32

Intravenous Fat Emulsions and the Pancreas: A Review *Andrew B. Leibowitz, Peggy O'Sullivan, and Thomas J. Iberti* 38

Synthetic Antiproteases in Acute Pancreatitis: An Experimental Study *Marek Dobosz, Zbigniew Sledziński, Andrzej Babicki, Piotr Juszkiewicz, Andrzej Basiński, and Zdzisław Wajda* 43

continued inside

Veterans Affairs

BETH
ISRAEL
MEDICAL
CENTER

THE MOUNT SINAI JOURNAL OF MEDICINE

The Mount Sinai Journal of Medicine is published by The Mount Sinai Medical Center of New York and has the following affiliates: Beth Israel Medical Center, New York; Bronx Veterans Affairs Medical Center, New York; and Elmhurst Hospital Center, New York.

Editor-in-Chief
Sherman Kupfer, M.D.

Editor Emeritus
Lester R. Tuchman, M.D.

Associate Editors
Harriet S. Gilbert, M.D. Julius Wolf, M.D.

Managing Editor
Claire Sotnick

Business and Production Assistant
Claudia N. Melendez

Assistant Editors

David H. Bechhofer, Ph.D.
Constanin A. Bona, M.D., Ph.D.
Edward J. Bottone, Ph.D.
Jurgen Brosius, Ph.D.
Lewis Burrows, M.D.
Joseph S. Eisenman, Ph.D.
Richard A. Frieden, M.D.
Steven Fruchtman, M.D.
Paul L. Gilbert, M.D.
James H. Godbold, Ph.D.
Richard S. Haber, M.D.
Noam Harpaz, M.D.
Dennis P. Healy, Ph.D.
Tomas Heimann, M.D.
Barry W. Jaffin, M.D.
Andrew S. Kaplan, D.D.S.
Samuel Kenan, M.D.
Suzanne Carter Kramer, M.Sc.
Mark G. Lebwohl, M.D.
Kenneth Lieberman, M.D.
Charles Lockwood, M.D.
Steven Markowitz, M.D.
Bernard Mehl, D.P.S.
Myron Miller, M.D.
Edward Raab, M.D.
Allan Reed, M.D.
Allan E. Rubenstein, M.D.
David B. Sachar, M.D.
Henry Sacks, M.D.
Robert Safirstein, M.D.
Ira Sanders, M.D.
Martin H. Savitz, M.D.
Clyde B. Schechter, M.D.
Michael Serby, M.D.
Phyllis Shaw, Ph.D.
George Silvay, M.D.
Barry D. Stimmel, M.D.
Nelson Stone, M.D.
Max Sung, M.D.
Rein Tideiksaar, Ph.D.
Richard P. Wedeen, M.D.
Michael F. Wesson, M.D.

Editorial Board

Barry Freedman, M.B.A.
Richard Gorlin, M.D.
Nathan Kase, M.D.
Panayotis G. Katsoyannis, Ph.D.
Charles K. McSherry, M.D.
Jack G. Rabinowitz, M.D.
John W. Rowe, M.D.
Alan L. Schiller, M.D.
Alan L. Silver, M.D.
Alvin S. Teirstein, M.D.
Rosalyn S. Yalow, Ph.D.

The Mount Sinai Journal of Medicine (ISSN No. 0027-2507; USPS 284-860) is published 6 times a year in January, March, May, September, October, and November in one indexed volume by the Committee on Medical Education and Publications (Owner), The Mount Sinai Hospital, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Subscription price: individuals, U.S. and Canada, $60 per year; all libraries, $65; individuals outside the U.S. and Canada, $70. Single copies, $15. New subscriptions begin with the first issue of the calendar year. Send notice of change of address 60 days before the change is effective. Second-class postage paid at New York, NY and at additional mailing offices. POSTMASTER: Send address changes to The Mount Sinai Journal of Medicine, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Copyright 1992 The Mount Sinai Hospital.

Statement of Ownership, Management, and Circulation filed with the U.S. Postal Service. Average no. copies each issue during preceding 12 months: 10a. net press run, 3354; 10b1. single copy sales, 530; 10b2. mail subscriptions, 2496; 10c. total paid or requested circulation, 3026; 10d. nonpaid distribution, 200; 10e. total distribution, 3226; 10f1. copies not distributed, 128; 10f2. return from news agents, none; 10g. total number of copies, 3354. Actual no. of copies of issue published nearest to filing date: 10a. net press run, 2723; 10b1. single copy sales, 30; 10b2. mail subscriptions, 2365; 10c. total paid or requested circulation, 2395; 10d. nonpaid distribution, 200; 10e. total distribution, 2595; 10f1. copies not distributed, 128; 10f2. return from news agents, none; 10g. total number of copies, 2723.

Volume 59
Number 1
January 1992

CONTENTS *continued*

Carcinoma of the Gallbladder: Review of the Literature and Report on 56 Cases at the Beth Israel Medical Center *Leigh H. Nadler and Charles K. McSherry* 47

An Outbreak of Bacteremic *Campylobacter jejuni* Infection *Wayne X. Shandera, Michael P. Tormey, and Martin J. Blaser* 53

Acute Myocardial Infarction in a Long-Term Care Institution for the Aged *Ivonne Z. Jimenez-Velazquez, Manuel Rodstein, Michael Mulvihill, Ming-Ann Hsu, Leslie S. Libow, and Richard R. Neufeld* 57

Screening Indices for Cytomegalovirus Retinitis in Patients with Human Immunodeficiency Virus *Gregory A. Butler and Alan H. Friedman* 61

PRELIMINARY REPORT

HIV-1 Seroprevalence in Pregnant Women Testing Positive on Serologic Screening for Syphilis *Rhoda S. Sperling, Michael Joyner, Joseph Hassett, Romina Kee, Patsy Wheatley, and Carlos Bauer* 67

CASE REPORTS

Phycomycosis (Mucormycosis) in Differential Diagnosis of Cerebral Mass Lesions in Intravenous Drug Users *Arthur A. Gaing, Francisco Corbalan, and Jesse Weinberger* 69

Cortical Blindness in Postpartum Preeclampsia Progressing to Eclampsia: Case Report *David Mandelkern and Andrew Burger* 72

Duodenocolic Fistula: An Unusual Presentation of Esophageal Squamous Cell Carcinoma *Petachia Reissman, Randolph M. Steinhagen, and Patrick F. Enright* 75

Hyperthyroidism in Patients Treated with Lithium: Report of Nine Cases *David K. Sirota, Rhoda H. Cobin, Walter Futterweit, Robert L. Segal, David R. Jacobs, and Edward Merker* 79

Ovarian Pregnancy with Ipsilateral Mature Cystic Teratoma of the Ovary: Case Report *Melissa Feltingoff, Debra S. Heller, and Ira J. Bleiweiss* 82

STUDENT CORNER

Familial Adenomatous Polyposis: Case Report and Review of Extracolonic Manifestations *Kenneth Alan Konsker* 85

Index to Advertisers

Eli Lilly and Company	93

please mention to advertisers that
you saw their advertisement in

The Mount Sinai Journal
of Medicine

From Bench To Bedside and Back

This article inaugurates a new feature: discussions of basic science for clinicians that ideally also feed back useful ideas from bedside to laboratory bench.

Lactic Acidosis, Ketoacidosis, and Energy Turnover:

"Figure" You Made the Correct Diagnosis Only When You Have "Counted" on It—Quantitative Analysis based on Principles of Metabolism

MITCHELL L. HALPERIN, M.D., FRCP(C), KAMEL S. KAMEL, M.D., FRCP(C), AND
SURINDER CHEEMA-DHADLI, PH.D.

Abstract

Three cases are presented to illustrate that quantitative analysis based on physiologic principles can help resolve certain controversies in clinical medicine. For example, in case 1, a patient with severe hypoxia, the rate of production of lactic acid is so high that only restoration of delivery of oxygen is rational therapy. If the degree of hypoxia exceeds 5.6% of demand, dichloroacetate will not lessen the degree of acidosis. Further, even when delivery of oxygen is returned to normal, the rate of fall in lactate and rise in bicarbonate in plasma will be relatively slow. In case 2, a patient with diabetic ketoacidosis, our discussion stresses that the rate of production of ketoacids is not that rapid and that the degree of ketoacidosis is influenced to a major degree by decreasing the rate of oxidation of ketoacids in brain and kidneys. Case 3, a patient with severe hyperglycemia, illustrates that insulin will only promote the oxidation of glucose at a rapid rate once the levels of fatty acids and ketoacids decline to low levels. Accelerated transport of glucose by insulin is only a permissive action for the oxidation of glucose.

OUR PURPOSE in this paper is to illustrate that a quantitative analysis that relies on simple biochemical and physiologic principles can go a long way to provide reasonable answers to questions which are currently unresolved. Three clinical cases have been selected to emphasize this point. To answer the questions posed by each case, discussion is divided into three sections: first, the pertinent principles of metabolism that apply to the case; second, under the title "Figure It Out," discussion of these principles in a quantitative fashion; third, under the title "Count on It," the material in the first two sections is utilized to answer the questions posed at the bedside. The specific clinical problems discussed and the questions they raise are:

Lactic Acidosis. Is it advisable to administer sodium bicarbonate or dichloroacetate (DCA) to a patient with lactic acidosis caused by an inadequate supply of oxygen? If the production of lactic acid stops and the concentration of lactate in blood is 15 mmol/L, by what amount might the concentration of lactate decline in the next 15 min?

Diabetic Ketoacidosis. How fast are ketoacids formed in a patient with diabetic ketoacidosis (DKA)? If sodium bicarbonate is to be adminstered, how much should be given, under what circumstances, and for what specific objectives?

Energy Turnover: Correction of Severe Hyperglycemia with Therapy. Why does the concentration of glucose fall in the first 6 hours after insulin is given to a patient with severe hyperglycemia? What does this imply about the impor-

---

From the Renal Division, St. Michael's Hospital, University of Toronto, Toronto, Canada. Address correspondence and reprint requests to M. L. Halperin, M.D., F.R.C.P(C), Professor of Medicine, St. Michael's Hospital, 38 Shuter Street, Toronto, Ontario, Canada, M5B 1A6.

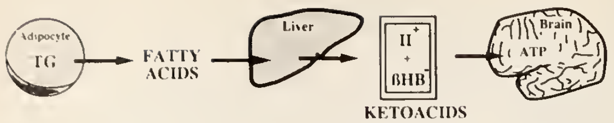


Fig. 1. Metabolic process; starting point is triglyceride in adipose tissue, product is ATP (and CO_2 + H_2O) in brain. Ketoacids (H^+ + β-HB^-) are produced in liver and removed by brain.

tant actions of insulin to promote the oxidation of glucose?

Lactic Acidosis

Case 1: Lactic Acidosis due to Shock. A 64-year-old man was admitted to the hospital because of crushing chest pain which lasted for about one hour. Blood pressure was hard to obtain, tachycardia was present, and his skin was cold and clammy. Severe metabolic acidosis, presumably lactic acidosis, was present (Table 1).

The question raised was, should sodium bicarbonate be administered to titrate H^+ ions produced during anaerobic metabolism, and if so, how much should be given? In addition, would dichloroacetate (DCA) help lessen the degree of acidosis? Affirmative and negative responses to each question were provided simultaneously and with vigor. What was to be believed?

Question 1:
Should bicarbonate be given to this patient?

Pertinent Principles of Metabolism

Production of H^+ Ions during Metabolism. H^+ ions are produced when a neutral substance (for example, glucose) is converted to an anion (as, for example, to lactate$^-$) (1). In this analysis, the net charge on adenine nucleotides (ADP, AMP, ATP) does not need to be considered because the conversion of ATP to ADP to do biological work initiates the regeneration of ATP via anaerobic glycolysis (equations 1–3) (2). Nevertheless, hydrolysis of ATP yields biological work and H^+ ions.

TABLE 1
Case 1: Laboratory Values

Plasma	Value
pH	7.10
Pco_2 (mm Hg)	20
Po_2 (mm Hg)	90
HCO_3^- (mmol/L)	10
Anion gap (MEq/L)	27

Decrease in bicarbonate from 25 to 15 mmol/L is equal to the increase in the anion gap ($Na^+ - Cl^- - HCO_3^-$) from 12 to 22 mEq/L. Thus this is a simple metabolic acidosis of the increased anion gap type, probably lactic acidosis. The degree of hyperventilation is in the appropriate range for this degree of metabolic acidosis (60).

$$\begin{aligned}
2\,ATP^{-4} &+ 2\,H_2O \rightarrow 2\,ADP^{-3} + 2\,Pi^{-2} \\
&+ 2\,H^+ + \text{Biological work} \qquad (1) \\
\text{Glucose} &+ 2\,ADP^{-3} + 2\,Pi^{-2} \rightarrow 2\,ATP^{-4} \\
&+ 2\,\text{Lactate}^- \qquad (2) \\
\text{Sum:}& \\
\text{Glucose} &\rightarrow 2\,\text{Lactate}^- + 2\,H^+ \\
&+ \text{Biological work} \qquad (3)
\end{aligned}$$

Stoichiometry of Generation of ATP and H^+ Ions. During aerobic metabolism, neutral fuels like glucose or triglycerides are oxidized to yield the uncharged products CO_2 and water. Hence there is no net production or removal of H^+ when ATP is regenerated. To generate 3 molecules of ATP, one atom of oxygen is consumed (P:O ratio is 3:1). Further, 12 mmol O_2 per minute are consumed to maintain the normal rate of utilization and regeneration of ATP (72 mmol/min). However, during anaerobic metabolism, the price paid is 72 mmol of H^+ ions for the 72 mmol ATP generated from 36 mmol glucose.

Buffering of H^+ Ions. Buffering of H^+ ions will result in the loss of bicarbonate and the titration of nonbicarbonate buffers in the body. Considering the limited buffer capacity of approximately 1000 mmol in a 70-kg adult (see appendix 1), it might seem rational to administer sodium bicarbonate in case 1.

Figure It Out

To determine if, and how much, bicarbonate might have to be given to this patient, one has to figure out the expected rate of production of H^+ during severe hypoxia. As discussed above, since 72 mmol of ATP must be produced per minute, the rate of production of H^+ during anoxia can be as high as 72 mmol/min (2).

Count on It

To titrate 72 mmol of H^+ ions/min with exogenous isotonic sodium bicarbonate, about 0.5 L would have to be infused per min. In a patient with congestive heart failure, this rate of administration of intravenous fluid would be disastrous. Thus arguments about whether to use or not to use sodium bicarbonate are not addressing the central issue. The important thing to do is to deliver an adequate quantity of oxygen to tissues, and quickly. Hence we shall not consider the pros and cons of using bicarbonate in the setting of shock, but rather to point out its limited value (see refs 3–8 for discussion).

There is an additional factor to consider if bicarbonate is given to this patient with inadequate tissue perfusion and lactic acidosis. If the 150 mmol of sodium bicarbonate was infused with 50 g of glucose (1 L of D_5W), these 275 mmol of glucose could be converted into as much as 550 mmol of H^+ + lactate$^-$ ions (equations 1–3), more than negating any acid-base benefit from the administered bicarbonate.

Question 2:
Is it advisable to use dichloroacetate to treat this patient's lactic acidosis?

Pertinent Principles of Metabolism

Approaches depending on Metabolic Processes vs Metabolic Pathway. To examine the stoichiometry of a series of metabolic reactions, it is only necessary to consider the substrates and the products of that sequence; we have called this approach a "metabolic process" type of analysis (2, 9). A metabolic process can span more than one organ (Fig. 1). In general, the starting points are dietary or stored fuels, and the final products are ATP or energy storage forms (glycogen, triglyceride).

One can determine if H^+ ions are produced or consumed in a metabolic process by a quantitative examination of the net charge of substrates vs products (1). H^+ ions accumulate if the net charge of products other than H^+ is less anionic than that of substrates. The converse is also true.

Maximum Rate of Production or Removal of H^+ Ions Depends on Rate of Turnover of ATP. Since the concentration of ADP is so low in cells (10), oxidation of fuels to regenerate ATP only can proceed in response to an initial conversion of ATP to ADP (9, 11–14). It follows that the rate of utilization of fuel is determined in part by the rate of consumption of oxygen. Different fuels and various mixtures of fuels can and do compete to be the source for ATP.

How Much Fuel Can Be Oxidized per Unit Time? The rate of flux through a metabolic pathway in energy metabolism can be limited by the supply of substrates (glucose or fat-derived fuels, O_2, ADP) or the activity of the enzymes that catalyze the flux. The concentrations of products also could influence this flux when the process is close to thermodynamic equilibrium. Since this is not the situation in oxidative pathways, the concentration of products will not influence flux unless these products alter the activity of a rate-controlling enzyme(s) in some specific fashion (inhibit them directly or indirectly).

The availability of substrates rarely influences the process unless their concentrations are extremely low relative to their Km for the rate-controlling step. In general, this situation applies to ADP. For example, the supply of O_2 is usually not a limiting factor, except in those disease states associated with a very low cardiac output, as in case 1, during exercise to exhaustion (requiring >160 mmol O_2/min) (15) and when living at very high altitudes (16). Another example where a supply of fuels is crucial is in organs like the brain which have specific requirements for certain fuels. The fuel menu for oxidation by the brain is limited to glucose and ketoacids (17). Should delivery of glucose be in short supply and ketoacids not available (for example in insulin overdose), failure to regenerate sufficient ATP in the brain can lead to confusion or coma. In muscle, fuels may not be present at a high enough concentration to meet demands in prolonged, severe exercise when fatty acids from adipose tissue cannot be delivered to mitochondria of myocytes at a rate fast enough to meet their needs. Since fatty acids are so sparingly soluble in water, they exist in the circulation bound to albumin. Moreover, there are many aqueous compartments that fatty acids must transverse between the capillary lumen and the inner mitochondrial membrane; hence the rate of delivery of fatty acids via the circulation is limited when high rates of consumption are required. Two examples of this limitation are during vigorous exercise and when the rate of ketogenesis is high. With respect to DKA, the delivery of fatty acids to the liver is close to 0.6 mmol/min (1.0 mmol/L plasma [18], 0.6 L plasma/L blood). Much less than the maximum of 0.6 mmol of fatty acids that are delivered can be extracted by the liver per min. The stoichiometry for conversion of palmitate to ketoacids (equally divided between β-hydoxybutyric and acetoacetic acids) is:

$$\text{Palmitate} + 6\ O_2 \rightarrow 4\ \text{Ketoacids} + 27\ \text{ATP} \quad (4)$$

With a maximum delivery of much less than 0.6 mmol palmitate/min, the availability of this substrate may limit ketogenesis to close to 1.6 mmol/min (4 × 0.4 mmol palmitate extracted per liter of blood) providing no oxidation occurs of the acetyl-CoA formed in the TCA cycle of hepatocytes.

Pyruvate Dehydrogenase Is the Key Enzyme in Energy Metabolism. Pyruvate dehydrogenase (PDH) is a key enzyme in the regulation of fuel metabolism (Fig. 2). It catalyzes the irreversible reaction in which pyruvate is converted to acetyl-CoA, which can no longer be converted back to glucose. Since carbohydrates and proteins must be preserved during fasting, flux through PDH is always tightly regulated (19–22). PDH is regulated by two mechanisms: first, its activity is inhibited by the products of the reaction it catalyzes (acetyl-CoA and NADH which are produced whenever fatty acids or ketoacids are oxidized); and second, the active form of PDH is reversibly inactivated via phosphorylation (20, 22–24). As a result of control of flux through PDH, a "fuel selection" hierarchy is set up whereby whenever fatty acids or ketoacids are being oxidized, there will be little if any flux through PDH (2). DCA maintains PDH in its active form by inhibiting its phosphorylation (23, 25, 26).

Figure It Out

To figure it out if DCA would be of value in the treatment of a patient with lactic acidosis, one has to consider what the expected rate of production of H^+ ions is. Consider an example: 11, but not the needed 12 mmol of oxygen are delivered to tissues of the body per minute, resulting in a deficit of oxygen of 1 mmol of oxygen per min (see appendix 2). One must also consider what the possible rate of removal of H^+ ions will be if lactate is the only fuel oxidized for the remaining consumption of O_2 (11 mmol/min).

Rate of Production of H^+ Ions. A deficit of 1 mmol of O_2 would mean that 6 mmol of ATP have to be produced via anaerobic glycolysis (P:O = 3:1, 2 atoms of oxygen in O_2). This would yield 6 mmol of H^+ ions per minute when glucose is the substrate (1 H^+ ion/ATP).

Rate of Removal of H^+ by Metabolism. How many mmol of lactate can be oxidized in a minute and how many mmol of lactate can be removed by glucogenesis in this setting? Metabolism of one mmol of lactate anions to neutral end-products (CO_2 or glucose) removes one mmol H^+.

Rate of oxidation of lactate and removal of H^+ ions: Three mmol of O_2 are required to oxidize 1 mmol of lactate plus one mmol of H^+ ion. Therefore a maximum of 3⅔ mmol of lactate and H^+ ions can be removed per minute with the available O_2 (equation 5).

$$\text{Lactate}^- + H^+ + 3\,O_2 \rightarrow 3\,CO_2 + 3\,H_2O \qquad (5)$$

Glucogenesis: The conversion of 1 mmol of lactate to 0.5 mmol of glucose requires the consumption of 3 mmol of ATP (equation 6).

$$\text{Lactate}^- + H^+ + 3\,\text{ATP} \rightarrow 0.5\,\text{Glucose} + 3\,\text{ADP} + 3\,\text{Pi} \qquad (6)$$

This metabolic sequence only occurs in the liver and kidneys. Therefore if 4.5 mmol of oxygen is delivered to the liver and kidneys each minute (about 40% of the available O_2), only 9 mmol of lactate would be removed in that time. This, however, requires that all the ATP produced by these organs be used to support glucogenesis, and none would be available for other metabolic work (e.g., pumping Na^+), an unlikely scenario. Moreover, the available experimental evidence indicates that DCA inhibits rather than stimulates glucogenesis in the liver (27).

Dichloroacetate. DCA has been shown to be effective in lowering the concentration of lactate in certain experimental models of lactic acidosis (28–32) and in some patients with lactic acidosis (33, 34). If DCA acts by activating PDH, it might "select" lactate as the preferential fuel to oxidize. Its benefit depends on how much fatty acid or glucose oxidation it "displaced" (a maximum of 4 mmol of lactate oxidation per min when 12 mmol O_2 are consumed/min, 3 O_2/lactate). DCA might also be beneficial in patients with lactic acidosis due to a low cardiac output if it led to an increase in the delivery of oxygen as a result of increasing the cardiac output. Although DCA has been shown to improve cardiac function in dogs with experimentally induced myocardial ischemia (35), to maintain the cardiac index in dogs with experimental lactic acidosis (31), and to increase myocardial efficiency index in patients with coronary artery disease (36), the data on the effect of DCA on the cardiac output in patients with lactic acidosis are less clear. While an effect of the drug to increase the cardiac output was suggested in a study by Stacpoole et al. (34), this was not substantiated in a more recent study (37).

Count on It

It is clear that DCA, at its best, can overcome only a very small degree of hypoxia (1/18th the usual rate of consumption of oxygen, or a reduction in the rate of consumption of oxygen of 5.6%). Said another way, complete oxidation of 1 lactate anion consumes only 1 H^+ ion while regenerating 18 ATPs, whereas anaerobic glycolysis yields 18 H^+ and 18 lactate anions per 18 ATPs. Thus oxidation of lactate must lose the H^+ battle if it is <18-fold greater than the rate of anaerobic glycolysis (5.6% hypoxia). This ability to withstand hypoxia is even less if fatty acids, glucose, or amino acids rather than lactate led to the regeneration of some of the ATP.

The above analysis, which suggests a limited role for activating PDH in the treatment of hypoxic lactic acidosis, seems to be in contradiction with the results of previous studies (28–32). Notwithstanding, conclusions from these studies were based on models where the rate of production of lactic acid was very low. For example, in the study by Graf et al. (28) on the effect of DCA in the treatment of hypoxic lactic acidosis in dogs, net rate of production of lactic acid was only 1 mmol/min. Since the rate of consumption of oxygen in dogs is close to 5 mmol/min (=30 mmol ATP/min), net rate of production of H^+ was very low, that is, equivalent to 3.3% hypoxia.

Question 3:

If the concentration of lactate is 15 mmol/L, by what amount might it decline in 15 min when oxygen delivery is adequate?

Figure It Out

Pool of Lactate. The volume of distribution of lactate can be assumed to be two-thirds of total body water for simplicity (38), or 30 L in a 70-kg person for this calculation. Thus the quantity of lactate present would be 450 mmol (30 liters × 15 mmol/L).

Rate of Removal of Lactic Acid. Oxidation of lactate could be as high as 4 mmol/min; glucogenesis in liver may utilize another 4 mmol/min of lactate. Therefore, with no further production of lactate, the decline in lactate (or rise in bicarbonate) in 15 min might be as much as 120 mmol in 30 L or 4 mmol/L. Since some organs will burn fuels other than lactate, less lactate will be oxidized; further, less than 100% of consumption of oxygen in the liver will be devoted to glucopaleogenesis (Fig. 3) (2).

Count on It

Even if there was no further production of lactic acid because the underlying defect was corrected, the fall in lactate in plasma will be much less than 3 mmol/L, because less than 100% of needed ATP will be supplied by lactate oxidation.

Diabetic Ketoacidosis

Case 2: Diabetic Ketoacidosis with Severe Hypokalemia during Therapy. A 33-year-old woman noted excessive thirst and polyuria in the past week, a new finding for her. Her condition deteriorated markedly in the past two or three days. Because of severe malaise, frequent vomiting, and a deteriorating mental state, she was brought to hospital.

On physical examination she responded only to painful stimuli; acetone was detected on her breath, and breathing was deep and rapid. Blood pressure was 70 mm Hg systolic, and the jugular venous pressure was below the sternal angle. No precipitating event was identified.

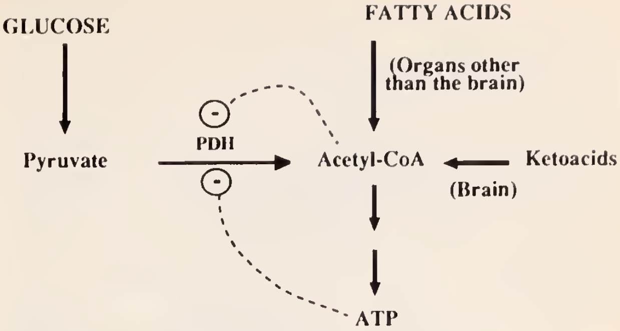


Fig. 2. Central role of pyruvate dehydrogenase (PDH). PDH catalyzes the irreversible conversion of pyruvate to acetyl-CoA. It is tightly regulated. In essence, oxidation of fatty acids leads to inhibition of PDH. *Reproduced* with permission from Halperin and Rolleston (ref. 2).

The key features of laboratory results (Table 2) are marked hyperglycemia, severe metabolic acidosis with an increased anion gap, and high concentrations of creatinine and urea in plasma.

Therapy in the first 4 hours consisted of in-

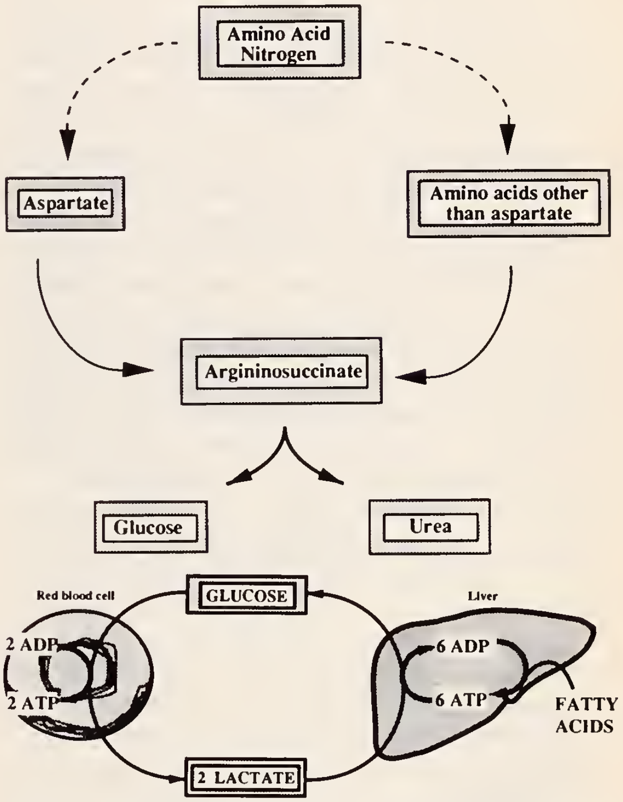


Fig. 3. Gluconeogenesis (above) vs glucopaleogenesis (below). Gluconeogenesis is the conversion of amino acids or glycerol to glucose. There is net gain of glucose for the body. In contrast, in glucopaleogenesis, glucose is merely catalytic in that there is neither net synthesis nor consumption of this metabolite; rather, energy trapped in ATP is transported from liver to red blood cells. Ultimate source of ATP regeneration is from oxidation of fatty acids in liver. *Reproduced* with permission from Halperin and Rolleston (ref. 2).

sulin (10 units IV bolus and 0.1 unit/kg body weight per hour). Intravenous fluid therapy consisted of 4 L of isotonic sodium chloride and 130 mmol KCl. One hundred mmol of sodium bicarbonate were given in the third hour because of persistent severe metabolic acidosis (pH 6.90, Pco_2 10 mm Hg, bicarbonate 2 mmol/L). A cardiac arrest occurred shortly thereafter; severe hypokalemia (2.1 mmol/L) was noted just prior to the arrest.

The questions to be addressed are, How fast might ketoacids be formed in DKA? Might an increased rate of ketogenesis explain why the condition of the patient deteriorated so markedly in the 2 days prior to admission? Should sodium bicarbonate be administered? What changes in therapy should be considered to avoid the cardiac arrest?

Question 1:
How fast might ketoacids be formed in diabetic ketoacidosis?

Pertinent Principles of Metabolism

Role of the Liver in Metabolism. The liver is the organ that initiates the metabolism of what we call unique fuels (ethanol, most amino acids, and so on) to produce universal fuels (glucose, ketoacids) (Fig. 4) (2, 9). Hence the liver is the only major site of production of ketoacids (39).

Rate of Turnover of ATP and the Production of H^+ Ions. Since the concentration of ADP is so tiny in cells (10), oxidation of fuels to regenerate ATP can only proceed at a rate which is linked to the rate of hydrolysis of ATP in those cells (9, 11, 13, 14). Since H^+ ions are produced in certain metabolic reactions that regenerate ATP, an upper limit on this rate will be set by the need to regenerate ATP in organs and organelles.

TABLE 2
Case 2: Values in Plasma during First Several Hours

Plasma	Admission Value
Glucose (mmol/L [mg/dL])	41 [738]
Urea (mmol/L [mg/dL])	10 [28]
Creatinine (μmol/L [mg/dL])	250 [2.9]
Na (mmol/L)	121
K (mmol/L)	4.0
Cl (mmol/L)	92
HCO_3 (mmol/L)	1.5
pH	6.80
Pco_2 (mm Hg)	10
Albumin (g/L)	26

Predominant acid-base lesion is severe metabolic acidosis of the increased anion gap type with expected degree of hyperventilation. The most likely basis for this acidosis is diabetic ketoacidosis.

Removal of H^+ Ions by Metabolic Processes. H^+ ions accumulate when the conjugate base of the acid is retained in the body or excreted in the urine with Na^+ or K^+ (1). The rate of oxidation of ketoacids will be determined by the rate of turnover of ATP in the major organs that oxidize them, brain and kidneys, and the availability of alternative fuels (e.g., oxidation of fatty acids in kidneys, Table 3) (13).

Figure It Out

The liver is the major site for production of ketoacids. Since ATP is produced in that process, the rate of turnover of ATP may set an upper limit on the rate of production of ketoacids (13, 14). The key facts are:

- When measurements of arteriovenous differences of oxygen and total hepatic blood flow were made, the rate of consumption of O_2 in the liver was close to 3000 mmol/day (40).
- Given the rate of appearance of urea during DKA, Jungas et al. (9) have calculated that approximately 600 mmol of O_2 will be consumed in the conversion of protein to glucose. Thus, the production of ketoacids will be supported by a consumption of 2400 mmol of oxygen.
- Since the oxidation of 1 mmol of palmitate consumes 6 mmol of O_2 and produces 4 mmol of ketoacids equally divided between β-hydroxybutyric acid and acetoacetic acid (equation 4), the maximal rate of production of ketoacids should be close to 1600 mmol/day.

Count on It

For ketoacidosis to worsen rapidly, either the rate of production of ketoacids must rise markedly or the rate of utilization of ketoacids must decline appreciably. Notwithstanding, there is little leverage to increase production appreciably because of the control exerted by the limited rate of turnover of ATP in hepatocytes. Perhaps a higher level of fatty acids in the circulation and an increased rate of turnover of ATP in the liver (e.g., enhanced conversion of lactate to glucose,

TABLE 3
Ketoacid Turnover during Chronic Fasting (mmol/day)

Ketoacid metabolism	Organ	Turnover
Net production	Liver	1500
Oxidation	Brain	750
	Kidney	250
	Muscle, etc.	200
Conversion to acetone	?	150
Excretion	Kidney	150

an ATP consuming reaction) could lead to some increase in the rate of ketogenesis. Nevertheless, the potential for an increased rate may be offset by a lower rate of delivery of fatty acids and oxygen (hypovolemia) and the increased production of ATP during the conversion of amino acids to glucose (9).

The degree of ketoacidosis can be influenced to a major degree by decreasing the rate of oxidation of ketoacids. The majority of ketoacids are oxidized to regenerate ATP in the brain and kidney (Table 4). The presence of coma can decrease the demand for the synthesis of ATP in the brain by 50% (41); this is equivalent to the failure to remove 375 mmol of ketoacids/day. Similarly, the very low GFR due to hypovolemia will decrease the rate of turnover of ATP in the kidney (less reabsorption of sodium) (12); this could diminish the oxidation of ketoacids by the kidney by 50% or 100 mmol/day. Both of these factors could lead to failure to oxidize several hundred mmol of ketoacids per day. Hence it is easy to understand why the natural history of DKA has a marked worsening with stupor and prerenal failure (an extra 375 + 100 mmol of ketoacids accumulate per day, and only close to 1000 mmol of H^+ can be buffered in vivo).

Question 2:
Should sodium bicarbonate be administered?

The primary goal is to reverse severe acidosis because it is considered to be life-threatening.

Pertinent Principles of Metabolism

The principles enumerated for case 1 apply.

TABLE 4
Rates of Production and Removal of H^+

H^+ ions	Rate (mmol/min)	Comments
Production		
Lactic acid		
Anoxia	72	Complete anoxia
	7.2	10% hypoxia
Ketoacids	1	Lack of insulin
Toxic alcohols	<1	Major threat: toxins, not H^+
Removal		
Kidney (by NH_4^+ excretion)	0.05 to 0.2	Has lag period
		Metabolic acidosis needed for rapid rates
Metabolism		
Lactate	4–8	Half by oxidation, half by glucogenesis
Ketoacids	0.8	Oxidized primarily in brain and kidneys

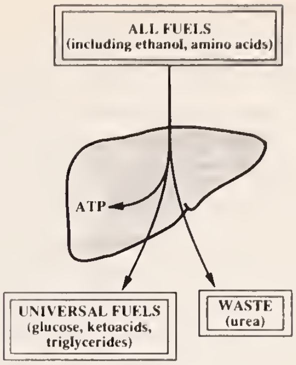

ALL FUELS
(including ethanol, amino acids)
ATP
UNIVERSAL FUELS
(glucose, ketoacids, triglycerides)
WASTE
(urea)

Fig. 4. Role of liver in metabolism of energy fuels. Liver is site of initial metabolism of a diverse but specialized group of fuels such as ethanol, most amino acids, and carbohydrates such as fructose. These fuels are converted to glucose, fatty acids, triglycerides, or ketoacids, "universal fuels" which can be utilized in a wide variety of organs. Hence, liver is the only obligatory site for synthesis of a large array of enzymes (consider alcohol dehydrogenase and ethanol metabolism). *Reproduced* with permission from Halperin and Rolleston (ref. 2).

Figure It Out

Raise the pH 0.3 Units. Since the antilog of 0.3 is 2, either the concentrations of bicarbonate must double or the P_{CO_2} halve to raise the pH 0.3 units. It is the relative (multifold), not the absolute change in bicarbonate that is important.

Production of CO_2. With a consumption of oxygen of 12 mmol/min and an RQ near 0.8, 9.6 mmol of CO_2 will be produced per min by metabolism. If one infuses sodium bicarbonate at a rate of 10 mmol per min during severe acidosis, almost all of it will be converted to CO_2. Hence the instantaneous rate of production of CO_2 will double. If there were no increase in alveolar ventilation, the P_{CO_2} would increase twofold.

Buffering of H^+ Ions by Nonbicarbonate Buffers. When 4 mmol of HCl/kg body weight was administered acutely to rats, the concentration of bicarbonate in plasma fell by 10 mmol/L and only 2% of H^+ ions were buffered by nonbicarbonate buffers (42). With much larger loads of H^+ ions, almost all the extra H^+ ions are buffered by nonbicarbonate buffers (43). Hence, during correction of very severe acidosis, almost all of the initial bicarbonate administered will be consumed during back-titration of nonbicarbonate buffers (44).

Count on It

If one accepts for the moment that very severe acidosis threatens the life of a patient, one should raise the concentration of bicarbonate and the pH of the blood when these values are too low. The question remains, at what pH and what concentration of bicarbonate is the life of the patient threatened?

Since these values are not clearly defined by clinical experience or experimental study, let us simply set an objective. If we try to raise the pH of blood from 6.8 to 7.1, what will our target be for the concentration of bicarbonate? Assuming that the Pco_2 of arterial blood remains constant at 10 mm Hg after the initial bolus of sodium bicarbonate, our target will be that the concentration of bicarbonate must double or rise from 1.5 mmol/L (Table 3) to 3.0 mmol/L. Notwithstanding, since there is so much H^+ ion buffering in the ICF and nonbicarbonate buffers at this pH (44), more than 100 mmol of bicarbonate will probably be needed to achieve this small absolute rise in bicarbonate. In reality, since most of the administered bicarbonate will be converted to CO_2, the Pco_2 might rise somewhat during the infusion if alveolar ventilation cannot increase appreciably. In this case, the concentration of bicarbonate will have to be somewhat higher to achieve the desired pH if endogenous acid production was not stimulated.

Question 3:
What changes in therapy should be considered to avoid cardiac arrest?

The most likely basis for the cardiac arrest was the extreme degree of hypokalemia (2.1 mmol/L at the time of the arrest). Factors contributing to this were the initial value for the concentration of K^+ in plasma (4.0 vs the expected 5.5 mmol/L [45]), and the two major factors which caused a shift of K^+ into cells (insulin and bicarbonate). To avoid serious hypokalemia, the following questions should be considered:

1. Might the administration of insulin be delayed to permit K^+ to be administered to replace some of the deficit of K^+? The key point is that insulin will only cause a modest decline in the rate of production of H^+ ions acutely but may cause a rapid decline in the concentration of K^+. If insulin were not given for one hour, how much more severe would the degree of metabolic acidosis become? Even if the oxidation of ketoacids were low, and all the ketoacids produced in the liver were retained, less than 100 mmol of ketoacids should accumulate in 60 min (production close to 1 mmol/min; Tables 3, 4). Hence if 100 mmol of bicarbonate were given in this hour, there should be little danger of aggravating the degree of acidosis.

2. How much K^+ should be administered? The amount of K^+ that must be given to prevent serious hypokalemia will not be known with certainty. If one would be willing to accept an upper limit of 2 mmol/L rise in the concentration of K^+ in the extracellular fluid (ECF), then one can safely administer 24 mmol of K^+ acutely (assume ECF volume is 12 L and no K^+ enters cells). Since the ECF was expanded by 2 L with K^+-free isotonic saline in hour 1, another 12 mmol of K^+ plus the 2 mmol of K^+ excreted in the urine (see appendix 3) can be given without a danger of severe hyperkalemia (total administration should be 38 mmol of K^+). In addition, some K^+ will enter cells to replace the intracellular deficit of K^+ and in response to the shift of H^+ ions from the intracellular fluid (ICF) when bicarbonate was administered. Therefore even more K^+ will have to be given in the first hour.

An appropriate way to do this will be to monitor the concentration of K^+ in plasma every 15 min early in therapy, increasing the amount of K^+ infused in response to low concentrations in plasma (46). It is tempting, knowing that conventional therapy was not ideal, to speculate that insulin could be withheld for 60 min or so to provide time to administer this amount of K^+. Nevertheless, one must have excellent malpractice insurance to do so (that is why we did not use the title “Count on It” at this juncture).

Correction of Severe Hyperglycemia With Therapy

Administration of insulin to a normal subject will lead to a fall in the concentration of glucose in blood. The most important actions of insulin to cause this fall are inhibition of the rate of production of glucose and acceleration of uptake and thereby oxidation of glucose. Although the rate of transport of glucose into cells with a sensitive transport system (muscle, adipose tissue) is augmented by insulin (47), we wish to examine whether this is a major action of insulin which leads to an accelerated rate of oxidation of glucose in all situations.

Case 3: Why Did the Concentration of Glucose Fall? A 42-year-old woman had chronic pancreatitis secondary to abuse of ethanol. She had several recent hospital admissions for problems related to hyperglycemia. In the past several days, she complained of excessive thirst and urination. To quench her thirst, she drank ice-cold apple juice (750 mmol of glucose/L). On admission, she was somewhat confused, but not comatose. Physical examination revealed a very modest degree of contraction of her ECF volume, and acetone was not detected on her breath. Respirations were normal, not rapid and deep. There were no other features of note. Urine output over the first six hours was 0.67 L. The important laboratory data are provided in Table 5.

A bolus of 6 units of insulin was given in the emergency room and a constant infusion of insulin (0.1 unit/kg body weight per hour) was started. She also received 4 L of saline (2 L of isotonic saline and 2 L of half-normal saline which contained 40 mmol KCl/L) as intravenous fluids.

Question:
Why did the concentration of glucose fall in the first six hours of therapy?

Pertinent Principles of Metabolism

Concentration of Glucose. The concentration of anything is the quantity of material per unit volume of fluid in which it is dissolved. Hence a quantitative analysis of a change in concentration of glucose will involve events changing the numerator (amount of glucose) and the denominator (volume of the ECF). To examine factors influencing the numerator, one must define events influencing the rate of production and removal of glucose. To examine factors influencing the denominator, changes in the ECF volume should be assessed, requiring for the most part an estimate of the positive or negative balance for sodium.

Rates of Metabolic Processes to Remove Glucose. The major metabolic processes to remove glucose are its conversion to stores (glycogen, triglycerides) or its oxidation to regenerate ATP. The rate of synthesis of glycogen requires a high level of insulin and glucose, plus time to synthesize the enzymes that catalyze the production of glycogen in liver and muscle. This is not a pathway with a rapid rate of flux in a setting where there is a relative deficiency of insulin.

The rate of synthesis of fatty acids from glucose is also a pathway with a slow rate of flux. Estimates of this rate are less than 0.5 g of glucose per hour in normal persons and even lower rates in fasting patients (48–50).

The limits for the rate of oxidation of fuels to yield ATP have been discussed in previous sections. In summary, the principles are:

Rate of turnover of ATP: The maximum rate of oxidation of fuels is set by the availability of ADP. Said another way, the rate of turnover of ATP sets this upper limit.

PDH rule: PDH is an essential step for the oxidation of glucose (Fig. 2). It is inhibited when fatty acid or ketoacids are oxidized.

Fuel selection: There are more fuels available to oxidize than are needed to make the requisite amount of ATP. Hence it is necessary to select which fuel should be oxidized. In general terms, fuels derived from fatty acids are oxidized in preference to carbohydrates (the PDH rule).

Synthesis of Glucose in the Liver. Glucose can be formed from the hydrolysis of glycogen or via gluconeogenesis. One should distinguish two physiologically different fluxes incorporated into the term gluconeogenesis, the conversion of protein (amino acids) to glucose (true gluconeogenesis), and the conversion of lactate to glucose (glucopaleogenesis) (2) (Fig. 3). The conversion of amino acids to glucose yields a net gain of glucose; urea is a product, and almost 2 mmol of urea are formed per glucose synthesized (see appendix 4). A minor substrate for the gluconeogenic pathway is glycerol.

Site of Regulation in a Metabolic Pathway. To examine where control is exerted in the metabolic process where the oxidation of glucose yields ATP, one need only look ultimately at enzymes that catalyze reactions far from thermodynamic equilibrium (hexokinase, phosphofructokinase-1, pyruvate dehydrogenase) (2, 10). To control flux, there must either be insufficient substrate relative to the km for that reaction in vivo or inhibition of that key enzyme in such a way that its maximum velocity is decreased.

Said another way, this Vmax type of inhibition means that there will be reduced flux in that pathway, no matter how high the concentration of its substrates. This type of control exists at two stages of the oxidation of glucose, at the hexokinase-phosphofructokinase-1 system of glycolysis, and at PDH (2, 10) (Fig. 2). This casts doubt on a major role for control of the oxidation of glucose by promoting its entry into myocytes. Since the hexokinase-phosphofructokinase-1 system which regulates glycolysis is controlled by signals re-

TABLE 5
Case 3: Laboratory Values

	Admission	6 hours later
Blood		
Glucose (mmol/L)	50	28
Bicarbonate (mmol/L)	10	12
β-HB (mmol/L)	10	6
Urea (mmol/L)	30	23
Urine		
Glucose (mmol/L)	—	300
Urea (mmol/L)	—	300
ECF volume (L)	12	16

ECF, extracellular fluid.
Volume of urine was 0.67 L over the first six hours of therapy.

lated to the availability of ADP (or surplus of ATP), and inhibition of hexokinase reduces its Vmax, there is very little if any opportunity to increase the rate of oxidation of glucose by increasing the transport of glucose into cells.

To accelerate the oxidation of glucose, less ATP must be synthesized from fat-derived fuels, or the rate of utilization of ATP must rise. It follows then that insulin augments the oxidation of glucose primarily by reducing the availability of fatty acids and ketoacids with a permissive effect of accelerated transport of glucose into these cells.

Figure It Out

Assessment of the Denominator. By calculating the positive balance for sodium and water, the increase in the ECF volume during therapy can be estimated. The initial volume of distribution of glucose was low in this patient owing to the contraction of her ECF volume. The initial ECF volume had probably declined from 15 L to 12 L (an estimate which can be evaluated by the chloride space [51]). To calculate the volume of distribution of glucose, one should add 4 L of ICF, that volume of ICF which does not require insulin for the transport of glucose in a 70-kg person (52). Thus a reasonable value for the volume of distribution of glucose before therapy would be 16 L. The quantity of glucose in her body is the product of its concentration (50 mmol/L) and its volume of distribution (16 L), or 800 mmol. Since the gain of ECF volume due to the intravenous infusion was 4 L, the new volume of distribution of glucose is 20 L.

Production of Glucose. Since almost 2 mmol of urea will be formed per mmol of glucose produced from protein (appendix 4), this rate of production of glucose can be calculated by examining the rate of appearance of urea. In adults with DKA, the rate of appearance of urea on admission in DKA is usually close to 0.3 mmol/min (53). In glucose terms, this is equivalent to close to 0.15 mmol/min or 0.027 g/min. The rate of appearance of urea was 40 mmol in 6 hours because 200 mmol of urea was excreted and the pool size for urea (total body water times the concentration of urea) declined by 160 mmol (36 L × 30 mmol/L at outset, vs 40 L × 23 mmol/L 6 hours later).

Glucose can also be produced from the glycerol moiety of triglyceride. Since glycerol is close to 10% of triglycerides by weight, and 200–250 g of fatty acids might be oxidized per day in an adult with DKA (1800 kcal), another 133 mmol (24 g) of glucose could be synthesized per day (0.1 mmol/min or 0.002 g/min) by this route.

Since production of glucose from glycogen is also low in this setting, the rate of production of glucose from endogenous sources is usually less than 0.5 mmol/min. Much larger inputs of glucose require an exogenous source of carbohydrate.

Removal of Glucose. Glucose can be removed by excretion in the urine or by metabolism.

Urine: During an osmotic diuresis induced by glucose, the concentration of glucose in the urine tends to be close to 300 mmol/L (53). Hence, knowing the volume of urine can reveal the quantity of glucose that was excreted in that time. The volume of urine excreted was two-thirds of a liter in 6 hours; hence 200 mmol of glucose was excreted.

Metabolism: Oxidation of glucose will only occur at a rapid rate once fatty acids and ketoacids are no longer readily available, as PDH is inhibited by the products of their oxidation. In this setting, fatty acids and ketoacids will be available for the 6-hour period (Table 5). As mentioned above, the conversion of glucose to stores will not occur at a rapid rate in this setting.

Count on It

Denominator Events. Without a change in the quantity of glucose, its concentration would have fallen from 50 to 40 mmol/L (800 mmol of glucose in 20 L) owing only to a denominator effect.

Numerator Events. *Urine:* Excretion of 200 mmol of glucose would result in a further fall in glucose to 30 mmol/L (800 − 200 mmol/20 L).

Metabolism: It appears that 20 mmol of glucose was produced from protein in this 6-hour interval. Since the concentration of glucose fell to 28 mmol/L, the metabolic actions of insulin were not responsible in a major way for the fall in the concentration of glucose in this setting.

Appendix 1
Buffering of H^+

Extracellular Fluid. The major buffer in the ECF is bicarbonate (43). Given its concentration (25 mmol/L) and the volume of the ECF (15 L in a 70-kg adult), ECF can buffer almost 375 mmol of H^+ ions (25 mmol/L fall in bicarbonate × 15 L).

Intracellular Fluid. The major buffers of the ICF are bicarbonate and histidines (in proteins and the dipeptides, carnosine and anserine [54]). Considering intracellular bicarbonate first, its pool size in the ICF is close to 330 mmol (11 mmol/L × 30 L) (55). With a fall in pH of the ICF of 0.3 units and a 50% fall in P_{CO_2} to 20 mm Hg, ¾ of this bicarbonate, or close to 250 mmol, will be titrated in very severe metabolic acidosis (42). With respect to histidines, the total content in the body is close to 2400 mmol in a 70-kg individual (close to 4% of the 50000 mmol of amino acids in the body plus those in carnosine and anserine). Since the pH of the ICF is close to the mean pK of histidine (56), there are 1200 mmol of potential H^+ ion binders. With a change in pH of 0.3 units, about 400 mmol of H^+ ions can be buffered via histidines.

One other point should be stressed. Organs like muscles which are designed to handle a large load of H^+ ions have more effective mechanisms to remove them than other organs (for example, hydrolysis of creatine phosphate [57], presence of histidine in dipeptides [54]). In contrast, brain will suffer most from a H^+ ion load in anoxia because it has a very high rate of anaerobic glycolysis in anoxia owing to its high metabolic rate and a low buffer capacity (56).

Inorganic phosphate is not an important buffer in the ICF or ECF because its concentration is too low (10).

To summarize, close to 1000 mmol of H^+ ions can be buffered, consisting of 300–350 mmol bicarbonate in the ECF, 250 mmol of bicarbonate in the ICF, and 400 mmol via intracellular histidines.

Appendix 2

How low must cardiac output be to cause lactic acidosis due to a deficit of 1 mmol O_2/min during hypoxia?

Tissues need about 12 mmol of oxygen/min at rest. Normally, the cardiac output is 5 L/min and each liter of blood contains close to 9 mmol of oxygen (140 g hemoglobin, molecular mass of hemoglobin is 66000, and the stoichiometry of 4 O_2/hemoglobin). Accordingly, 45 mmol of oxygen are delivered at rest per min (5 L/min × 9 mmol oxygen/L). Consequently, the cardiac output must be reduced to less than one-third normal (delivery of 12 mmol of oxygen/min) or 1.67 L/min, to obligate inadequate delivery of oxygen at rest, assuming a normal value for hemoglobin.

Appendix 3

Excretion of K in urine

Given a urine flow rate of 1–2 mL/min, only 0.1 L of urine will be produced in one hour. The expected concentration of K^+ in the urine is close to 20 mmol/L (58). Hence only 2 mmol of K^+ (20 mmol/L × 0.1 L) should be excreted.

Appendix 4

Relationship between production of glucose and urea

Nitrogen makes up 16% of most animal proteins. Since the atomic mass of nitrogen is 14 and urea contains two nitrogens, about 570 mmol of urea will be formed from 100 g of protein. Since some of the amino acids are ketogenic and a portion of the carbon must be released as CO_2 during gluconeogenesis, only about 60% or 60 g is available for conversion to glucose (333 mmol) (59). Thus the ratio of urea/glucose production is close to 1.7 in mmol terms.

References

1. Halperin ML, Jungas RL. Metabolic production and renal disposal of hydrogen ions. Kidney Int 1983; 24:709–713.
2. Halperin M, Rolleston F. Biochemical detective stories: a problem based approach to clinical cases. Burlington, NC: Neil Patterson Publications, 1990.
3. Cohen R, Iles R. Bicarbonate therapy and lactic acidosis. Diabetes Care 1984; 7:509–510.
4. Hindman B. Sodium bicarbonate in the treatment of subtypes of acute lactic acidosis: physiologic considerations. Anesthesiology 1990; 72:1064–1076.
5. Narins R, Cohen J. Bicarbonate therapy for organic acidosis: the case for its continued use. Ann Int Med 1987; 106:615–618.
6. Stacpoole P. Lactic acidosis: the case against bicarbonate therapy. Ann Int Med 1986; 105:276–279.
7. Ayus J, Krothapalli R. Effect of bicarbonate administration on cardiac function. Am J Med 1989; 87:5–6.
8. Graf H, Arieff A. The use of bicarbonate in the therapy of organic acidosis. Intens Care Med 1986; 12:285–288.
9. Jungas RL, Halperin ML, Brosnan JT. Lessons learnt from a quantitative analysis of amino acid oxidation and related gluconeogenesis in man. Physiol Rev 1992 (in press).
10. Voet D, Voet JG. Biochemistry. New York: John Wiley and Sons, 1990.
11. Halperin ML, Jungas RL, Pichette C, Goldstein M. A quantitative analysis of renal ammoniagenesis and energy balance: a theoretical approach. Can J Physiol Pharmacol 1982; 60:1431–1435.
12. Halperin ML, Vinay P, Gougoux A, Pichette C, Jungas RL. Regulation of the maximum rate of renal ammoniagenesis in the acidotic dog. Am J Physiol 1985; 248:F607–F615.
13. Halperin M, Cheema-Dhadli S. Renal and hepatic aspects of ketaocidosis: a quantitative analysis based on energy turnover. Diabetes/Metabol Rev 1989; 5:321–336.
14. Flatt J. On the maximal possible rate of ketogenesis. Diabetes 1972; 21:50–53.
15. Margaria R, Cerretelli P, Aghemo P, Sass J. Energy cost of running. J Applied Physiol 1963; 18:367–370.
16. West J. The 1988 Stevenson Memorial Lecture. Physiological responses to severe hypoxia in man. Can J Physiol Pharmacol 1989; 67:173–178.
17. Owen O, Morgan A, Kemp H, Sullivan J, Herrera M, Cahill GJ. Brain metabolism during fasting. J Clin Invest 1967; 46:1589–1595.
18. Owen O, Caprio S, Reichard GJ, Mozzoli M, Boden G,

Owen R. Ketosis of starvation: a revisit and new perspectives. Clin Endocrinol Metab 1983; 12:359–379.

19. Randle P. Fuel selection in animals. Biochem Soc Trans 1986; 14:799–806.
20. Patel M, Roche T. Molecular biology and biochemistry of pyruvate dehydrogenase complexes. FASEB J 1990; 4:3224–3233.
21. Fields A, Cheema-Dhadli S, Wolman S, Halperin M. Theoretical aspects of weight loss in patients with cancer: possible importance of pyruvate dehydrogenase. Cancer 1982; 50:2183–2188.
22. Denton R, Halestrap A. Regulation of pyruvate metabolism in mammalian tissues. Essays Biochem 1979; 15:37–77.
23. Whitehouse S, Randle P. Activation of pyruvate dehydrogenase in perfused rat heart by dichloroacetate. Biochem J 1973; 134:651–653.
24. Reed L. Pyruvate dehydrogenase complex. In: Horecker B, Stadtman E, eds. Current topics in cellular regulation. New York: Academic Press, 1969, 233–251.
25. Stacpoole P. The pharmacology of dichloroacetate. Metabolism 1989; 38:1124–1144.
26. Whitehouse S, Cooper R, Randle P. Mechanism of activation of pyruvate dehydrogenase by dichloroacetate and other halogenated carboxylic acids. Biochem J 1974; 141:761–774.
27. Stacpoole P. Effects of dichloroacetate on gluconeogenesis in isolated rat hepatocytes. Metabolism 1977; 26:107–116.
28. Graf H, Leach W, Arieff A. Effects of dichloroacetate in the treatment of hypoxic lactic acidosis in dogs. J Clin Invest 1985; 76:919–923.
29. Holloway P, Alberti K. Reversal of phenformin-induced hyperlactataemia by dichloroacetate in normal and diabetic rats. Diabetologia 1975; 11:350–351.
30. Johnson G, Alberti K. The metabolic effects of sodium dichloroacetate in experimental hepatitis in the rat. Biochem Soc Trans 1977; 5:1387–1389.
31. Park R, Arieff A. Treatment of lactic acidosis with dichloroacetate in dogs. J Clin Invest 1982; 70:853–862.
32. Abu Romeh S, Tannen R. Therapeutic benefit of dichloroacetate in experimentally induced hypoxic lactic acidosis. J Lab Clin Med 1986; 107:378–383.
33. Stacpoole P. The case against bicarbonate therapy. Ann Intern Med 1986; 105:276–278.
34. Stacpoole P, Harman E, Curry S. Treatment of lactic acidosis with dichloroacetate. N Engl J Med 1983; 309:390–396.
35. Mjos O, Miller N, Riemersma R, Oliver M. Effects of dichloroacetate on myocardial substrate extraction, apicardial ST-segment elevation, and ventricular blood flow following coronary occlusion in dogs. Cardiovasc Res 1976; 10:427–436.
36. Wargovich T, MacDonald R, Hill J, Feldman R, Stacpoole P, Pepine C. Myocardial metabolic and hemodynamic effects of dichloroacetate in coronary artery disease. Am J Cardiol 1988; 61:65–70.
37. Stacpoole P, Lorenz A, Thomas R, Harman E. Dichloroacetate in the treatment of lactic acidosis. Ann Int Med 1988; 108:58–63.
38. Roos A. Intracellular pH and distribution of weak acids across cell membranes: a study of D- and L-lactate and of DMO in rat diaphragm. J Physiol 1975; 249:1–25.
39. Robinson A, Williamson D. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol Rev 1980; 60:143–187.
40. Brauer RW. Liver circulation and function. Physiol Rev 1965; 43:115–213.
41. Kety SS, Polis BD, Nadler CS, Schmidt C. The blood flow and oxygen consumption of the human brain in diabetic acidosis and coma. J Clin Invest 1948; 27:500–510.
42. Vasuvattakul S, Warner L, Halperin M. Quantitative role of the intracellular bicarbonate buffer system in response to an acute acid load. Am J Physiol 1992 (in press).
43. Swan RC, Pitts RF. Neutralization of infused acid by nephrectomized dogs. J Clin Invest 1955; 34:205–212.
44. Fernandez P, Cohen R, Feldman G. The concept of bicarbonate distribution space: the crucial role of body buffers. Kidney Int 1989; 36:747–752.
45. Beigelman P. Potassium in severe diabetic ketoacidosis. Am J Med 1973; 54:419–420.
46. Donnelly S, Carlisle E, Kamel K, Halperin M. Hypokalemia. In: Kassirer J, ed. Current Therapy in Internal Medicine. Philadelphia: BC Decker 1990, 1015–1022.
47. Klip A. Regulation of glucose transport by insulin and non-hormone factors. Life Sci 1982; 31:2537–2548.
48. Acheson KJ, Flatt J-P, Jequier E. Glycogen synthesis versus lipogenesis after a 500-g carbohydrate meal. Metab Clin Exp 1982; 31:1234–1240.
49. Bjorntorp P, Sjostrom L. Carbohydrate storage in man: speculations and some quantitative considerations. Metabolism 1978; 27:1853–1885.
50. Hellerstein MK, Christiansen M, Kaempfer S, et al. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. J Clin Invest 1991; 87:1841–1852.
51. Tannen RL, Bleich HL, Schwartz WB. The renal response to acid loads in metabolic alkalosis; an assessment of the mechanisms regulating acid excretion. J Clin Invest 1966; 45:562–572.
52. Roscoe J, Halperin M, Rolleston F, Goldstein M. Hyperglycemia-induced hyponatremia: metabolic considerations in calculation of serum sodium depression. CMA J 1975; 112:452–453.
53. West M, Marsden P, Singer G, Halperin M. A quantitative analysis of glucose loss during acute therapy for the hyperglycemia hyperosmolar syndrome. Diabetes Care 1986; 9:465–471.
54. Davey C. The significance of carnosine and anserine in striated skeletal muscle. Arch Biochem Biophysics 1960; 89:303–308.
55. Conway EJ, Fearon PJ. The acid-labile CO2 in mammalian muscle and the pH of the muscle fibre. J Physiol (London) 1944; 103:274–289.
56. Roos A, Boron W. Intracellular pH. Physiol Rev 1981; 61:296–434.
57. Sahlin K, Harris R, Hultman E. Creatine kinase equilibrium and lactate content compared with muscle pH in tissue samples after isometric exercise. Biochem J 1975; 152:173–180.
58. West ML, Magner PO, Richardson RMA. A renal mechanism limiting the degree of potassium loss in severely hyperglycemic patients. Am J Nephrol 1988; 8:373–378.
59. Wolman SL, Fields ALA, Cheema-Dhadli S, Halperin M. Protein conversion to glucose: An evaluation of the quantitative aspects. J Parent Ent Nutrition 1980; 4:487–489.
60. Halperin M, Goldstein M. Fluid, electrolyte and acid-base emergencies. Philadelphia, PA: WB Saunders, 1988.

Grand Rounds

Why Kidneys Fail

Lance D. Dworkin, M.D.

The annual incidence of end-stage renal disease in the United States is approximately 100 cases per million among whites and about 2½ times that, or 250 per million, in blacks (1). Although this incidence may not seem great compared to other illnesses considered to be major public health problems, patients with end-stage renal disease may survive for many years; thus, in 1986, approximately 85,000 patients were undergoing chronic dialysis, at a cost per patient of $25,000 per year. The average cost of caring for a patient with a functioning renal transplant is significantly less, about $8,000 per year, but combined with the cost of the transplant procedure itself, is still considerable. In 1986, the total cost of the end-stage disease program in this country approached $3 billion per year, a significant portion of our nation's health care dollars. Because the number of patients with end-stage renal disease is increasing each year, the best hope for reducing the enormous costs of this disease is to develop therapies which will prevent patients from developing chronic renal failure.

The Progressive Nature of Renal Disease

The problem we face is that renal function declines progressively in all patients (2, Fig. 1). A remarkable feature of the process of this decline is that, while the rate at which renal function declines may vary considerably between patients who share a particular diagnosis, for individual patients, that rate is linear. The regular nature of this process suggests that there exists a secondary process perpetuating this loss of renal function which is common to all types of renal disease (Fig. 2). The cycle is set in motion by a primary renal disease that irreversibly damages a significant percentage of nephrons and produces an initial loss in kidney function. The kidney adapts to this loss of function with a variety of alterations that appear beneficial in the short term because they tend to return total kidney function toward normal. However, evidence I review below suggests that these adaptations may, in the long term, be harmful and promote further loss of kidney function. As additional nephrons are damaged secondarily, more extreme adaptations occur. In this way the cycle is closed, and patients progress to end-stage renal failure.

Compensatory adaptations of the kidney to a partial loss of function that have been related to progressive renal failure include:

- Increased glomerular pressure
- Glomerular hypertrophy
- Increased production of ammonia
- Increased oxygen consumption
- Increased phosphate excretion

A number of studies suggest that alterations in the perfusion of the glomerular capillaries promote glomerular injury. The hemodynamic alteration most closely linked with glomerular sclerosis is an increase in glomerular capillary pressure. More recent evidence suggests that the process of renal hypertrophy, the compensatory kidney growth which occurs in damaged kidneys, may also promote injury. In addition, a variety of metabolic and excretory functions which are altered in damaged kidneys have also been related to renal injury. These include increased oxygen consumption, possibly resulting in the generation of oxygen free radicals; increased ammoniagene-

---

Adapted from the author's presentation of Grand Rounds in Medicine at the Mount Sinai Medical Center on March 6, 1990. From the Department of Medicine, New York University Medical Center. Address reprint requests to the author, who is Associate Professor of Medicine, New York University Medical Center, 550 1st Avenue, New York, N.Y. 10016.

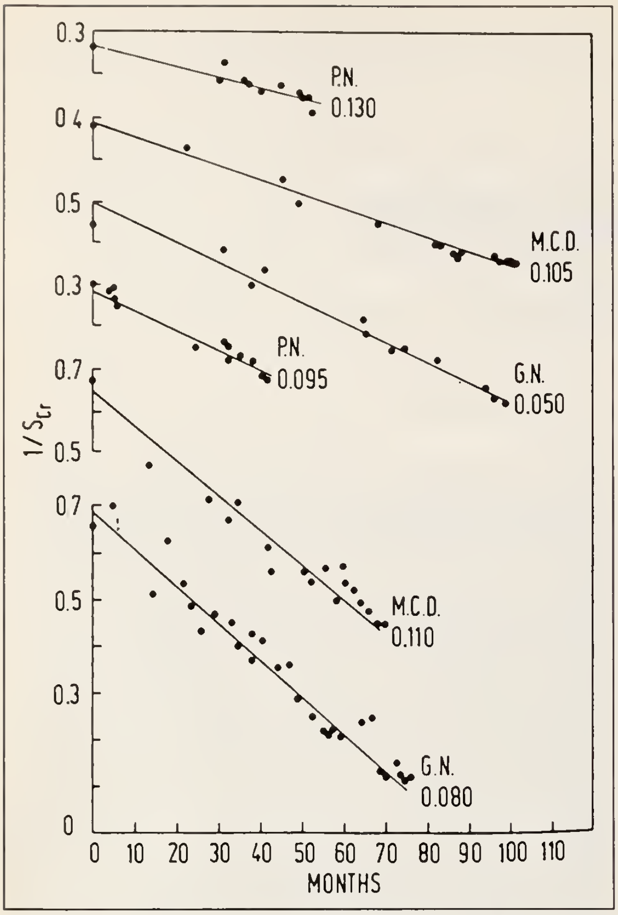


Fig. 1. Plot of reciprocal serum creatinine (in mg/dL) versus time in 6 patients with chronic renal disease. P.N., pyelonephritis; G.N., glomerulonephritis; M.C.D., medullary cystic disease. *Adapted* from reference 2 with permission.

sis, which may activate the complement by an alternative pathway; and increased phosphate excretion, which may promote calcification of the kidney. I concentrate here on the first two, for which I think the most evidence has been gathered.

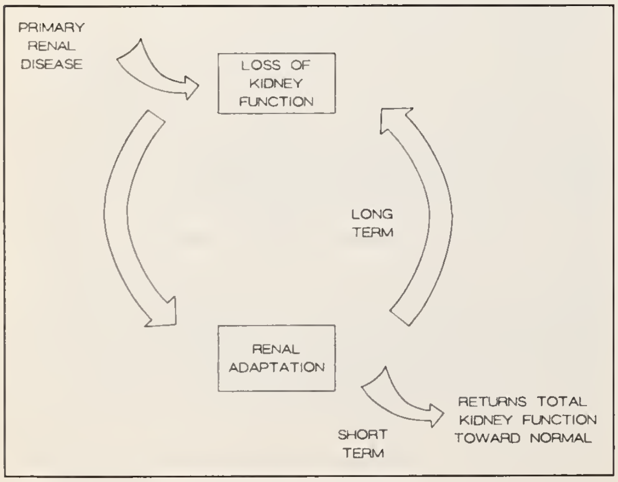


Fig. 2. Hypothesis relating compensatory adaptations to an acute insult with chronic, progressive kidney damage.

Role of Hemodynamic Factors

To examine the relationship between alterations in glomerular perfusion and glomerular injury, investigators have relied on micropuncture experiments in which structures on the surface of the kidney are punctured with glass pipets. This technique enables one to examine the glomerular filtration process and to precisely measure the four parameters that determine the ultrafiltration rate of water: the glomerular plasma flow rate, Q_A; the glomerular transcapillary hydraulic pressure difference, ΔP (the hydraulic pressure gradient across the glomerular capillary, equal to the difference between the hydraulic pressure within the glomerular capillary, P_{GC}, and the corresponding hydraulic pressure in Bowman's space or tubular pressure P_T); π_A, the oncotic pressure of the blood which opposes the movement of water from the lumen to the urinary space; and the ultrafiltration coefficient, K_f, the hydraulic conductivity of glomerular capillary wall averaged over the surface area of that capillary bed.

My colleagues and I have applied these techniques to experimental models of systemic hypertension, notably the spontaneously hypertensive rat (SHR). This is a strain genetically preset to develop severe, sustained hypertension by about 3 months of age. The usual control strain for the SHR is the Wistar Kyoto (WKY) rat. For the purposes of our study, note that SHR not only provide a model of hypertension but also a model of kidney disease. At about 1 year of age, SHR with 2 kidneys develop glomerular sclerosis which is largely limited to juxtamedullary nephrons (3).

Because glomerular hemodynamics can only be assessed in superficial nephrons by micropuncture techniques, it is difficult to make correlations between glomerular perfusion and injury in these animals. To overcome this limitation, I modified this model by removing one kidney (4). In SHR, uninephrectomy accelerates the pace of the renal disease, so that glomerular sclerosis is present after only 6 months, and also alters the distribution of the lesions to involve both superficial and deep nephrons. In SHR with unilateral nephrectomy, proteinuria becomes marked by the time the animals are about 6 months of age (Fig. 3). WKY rats, which are not hypertensive, do not develop proteinuria even in response to unilateral nephrectomy. In SHR that have been uninephrectomized, protein restriction prevents the development of proteinuria and glomerular sclerosis. In addition, the beneficial effect of protein restriction was independent of alterations in systemic blood pressure, which was similarly elevated in SHR fed the normal or the low-protein diet.

The mechanism by which glomerular sclerosis developed in uninephrectomized SHR was suggested by micropuncture studies in which the determinants of GFR were measured in these rats. Despite severe systemic hypertension, superficial glomerular hemodynamics are essentially normal in SHR with 2 kidneys, consistent with the finding that sclerosis does not develop in superficial nephrons of intact animals. However, when one kidney is removed from an SHR, two things happen (4). There is an increase in glomerular capillary plasma flow and, also, in the hydraulic pressure gradient across the glomerular capillary wall.

It is this pattern of hyperperfusion that is associated with injury, not only in uninephrectomized SHR, but also in other models of progressive glomerular sclerosis, including rats after significant surgical ablation of renal mass (5), the remnant kidney (RK) model, with mineralocorticoid-salt (DOC-SALT) hypertension (6) or with experimental diabetes mellitus (7).

That hyperperfusion actually causes the injury is supported by an examination of the effects of protein restriction, which reduces renal injury in these models, on glomerular hemodynamics. In each of the models mentioned above, protein restriction caused an increase in renal vascular resistance and reduced both glomerular plasma flow and pressure to normal. In every case, when perfusion was reduced, glomerular sclerosis was ameliorated.

Based on these observations, the general hypothesis presented in Fig. 2 can be modified to state that, in response to a primary renal disease and reduction in nephron number, glomerular capillary pressure and flow increase. Initially, this change in perfusion serves to increase the filtration rate in the surviving nephrons and return total GFR towards normal. However, in the long term, this increase in perfusion promotes glomerular sclerosis and a further reduction in nephron number. This process can progress to global glomeruli sclerosis.

Does Hemodynamically Mediated Glomerular Injury Occur in Humans? I suggest that the same phenomena that promote glomerular sclerosis in rat models of hypertension may also occur in a variety of forms of clinical renal disease, including unilateral agenesis, cortical necrosis, reflux nephropathy, hypertension, and even normal aging. Unilateral agenesis is a term applied to those few individuals born with just one kidney. It has been shown, in both autopsy and surgical series (8), that patients with unilateral agenesis have an increased incidence of focal glomerular sclerosis (Fig. 4). Increased glomerular size in patients with agenesis is associated with an increase in single nephron glomerular filtration rate (SNGFR). Although we cannot actually measure SNGFR in humans, we know that it is increased because, even though individuals with unilateral agenesis have half the normal number of nephrons, their total GFR is not different from that in persons born with two kidneys. By necessity, the increase in SNGFR must result from increased perfusion of these nephrons, similar to that observed in animals after removal of one kidney. Logically, therefore, the increased incidence of glomerular sclerosis in patients with unilateral agenesis might result from increased glomerular perfusion.

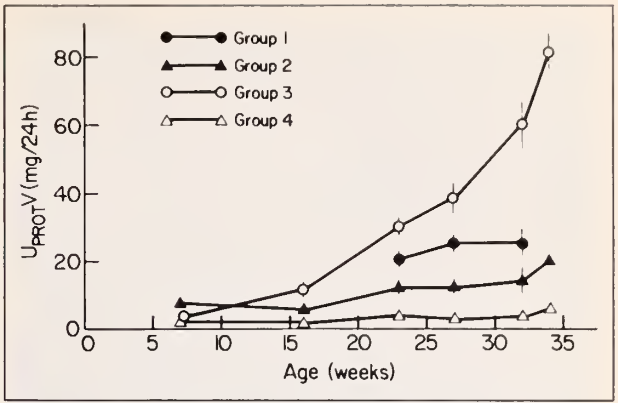


Fig. 3. Protein excretion versus time in spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats. Group 1, SHR with 2 kidneys; Group 2, WKY that had unilateral nephrectomy (UNX) at 6 weeks of age. Group 3, UNX SHR; Group 4, UNX SHR fed a low-protein diet. *Adapted* from ref. 4 with permission.

Unilateral agenesis is a relatively uncommon condition; however, a similar pathophysiologic sequence may apply to patients who recover from bilateral cortical necrosis. Rather than the usual reversible form of acute renal failure, these individuals suffer from irreversible necrosis of a significant portion of their renal cortex. As in other forms of acute renal failure, these individuals have very marked elevations of BUN at the time of presentation, and, with time, often recover enough renal function so that dialysis is no longer required. Nevertheless, long-term follow-up reveals that many ultimately develop a second increase in BUN and again require renal replacement therapy (9). To explain the late recurrence of renal failure in this population, it has been proposed that the initial necrosis is followed by adaptive increases in perfusion and in the filtration rates of the surviving nephrons. This hyperperfusion accounts for the early improvement in GFR; however, it is also responsible for the eventual development of glomerular sclerosis and end-stage renal failure in these patients.

Another clinical setting in which hyperperfusion has been suggested to promote progressive

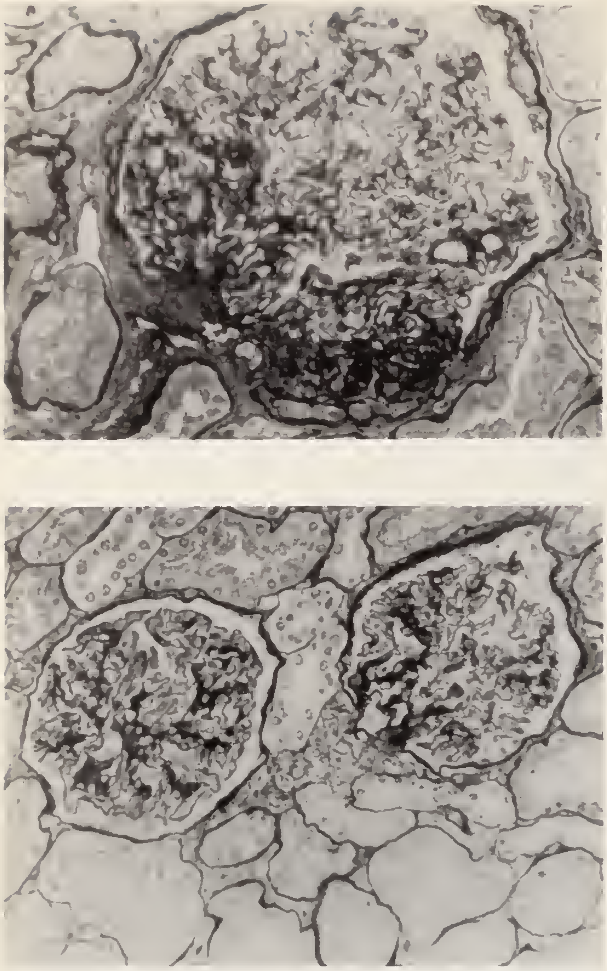


Fig. 4. Renal tissue from (top) 27-year-old man with unilateral renal agenesis and (bottom) 27-year-old normal control at identical magnification (×390). Both glomeruli and tubules are enlarged in patient with unilateral agenesis. Lower segment of this glomerulus shows early sclerosis. *Adapted* from ref. 34 with permission.

kidney damage is reflux nephropathy. In some young children, vesico-ureteral reflux can be severe and can result in scarring of the kidney and a substantial loss of nephrons. However, even in patients with severe disease, reflux often resolves spontaneously. In other individuals it is surgically corrected by reimplanting the ureters into the bladder. Despite the fact that demonstrating continued reflux or signs of infection may be difficult or impossible, a proportion of these patients go on to develop end-stage renal disease with advancing age (10).

The renal disease they develop is also surprising in that it seems to involve the glomeruli. In fact, the presence of proteinuria is a marker for those patients at risk of developing end-stage renal disease. In addition, when renal tissue from these patients is examined histologically, glomerular sclerosis is found. Thus, although the initial damage to the kidney in this case is interstitial, the process which ultimately destroys the kidney is glomerular. I suggest that end-stage renal disease in these patients is caused by the same hemodynamic changes that follow surgical reduction in renal mass in rats. The initial damage, caused by reflux and infection, is followed by adaptive increases in glomerular perfusion which promote the late development of proteinuria, glomerular sclerosis and end-stage renal disease.

Recently it has been suggested that patients with mild to moderate essential hypertension may be at risk for developing end-stage renal disease. In support of this hypothesis, Rostand et al. (11) performed a retrospective analysis of 94 patients treated for hypertension. Patients with diabetes, clinically significant proteinuria, pathologic elevations in serum creatinine, secondary causes of hypertension, and known renal disease were excluded from the analysis. The researchers found that, after an average follow-up period of 58 months, 15% of patients had a significant deterioration in renal function, defined as an increase in serum creatinine of greater than 0.4 mg/dL. Factors associated with an increased risk for a decline in renal function were advanced age, black race, and a higher number of missed office visits.

Although these data fail to establish that mild hypertension can lead to end-stage renal disease, they do suggest that a significant number of patients being treated only for essential hypertension are at risk of losing renal function. The factors responsible for a decline in GFR in this population are uncertain; however, it has been suggested that hypertensive nephrosclerosis is hemodynamically mediated and results from transmission of the increased systemic pressure to the glomerular capillaries.

In fact, hemodynamically mediated glomerular injury may occur in all of us. It has long been recognized that glomerular filtration rate in humans declines with advancing age (12), and that this decline is associated with progressive increase in the number of sclerotic glomeruli (13). It has been suggested (14) that the glomerular sclerosis of aging is a consequence of the "normal" pressures and flows to which our glomeruli are exposed daily. In fact there is no true "normal" GFR. GFR is largely determined by the amount of protein in the diet, and values for GFR considerably lower than that considered to be normal have been observed in individuals consuming a low protein diet (15). Therefore, were we all to eat a diet that was less rich in protein, we might see a lesser decline in GFR with aging. Most often, the lower GFR in elderly individuals is of little clinical significance. However, if an older individual develops a primary renal disease, the combined loss of nephrons resulting from the aging process and the renal disease might increase the risk of developing end stage renal disease.

Therapies to Prevent Progressive Kidney Damage

So far, I have suggested a number of clinical situations in which the same processes that damage rat kidneys after reduction in renal mass may operate. In fact, the diseases I have mentioned represent only a few of the renal diseases in which hemodynamic alterations may promote progressive kidney failure. The obvious question is, can anything be done to alter this process and reduce the incidence of ESRD?

Protein Restriction. In the rat studies reviewed above, protein restriction was extremely effective in reversing the hemodynamic alterations which promote glomerular disease and preventing kidney damage. Data have been obtained which suggest that protein restriction may have similar effects in humans (16). However, there has not yet been a sufficiently large, randomized, prospective, controlled trial that clearly establishes the efficacy of protein restriction. In fact, a large, multicenter trial of protein restriction in patients with chronic renal disease, the Modification of Diet in Renal Disease study, is currently being sponsored by the National Institutes of Health. Presumably, this study will provide a conclusive answer to the question in the next few years.

However, there are several potential problems with protein restriction as standard therapy for large numbers of patients with chronic renal disease. First, the degree of protein restriction necessary to produce a beneficial effect is probably quite marked. This low-protein diet is a radical departure from our standard western fare and, therefore, will be difficult for patients to tolerate and sustain. In addition, patients will have to remain on the diet for the rest of their lives. This is required because, as soon as one resumes a normal protein intake, glomerular perfusion will rise and the rate of decline in GFR accelerate. Finally, this manipulation is likely to be most useful when applied to individuals with only moderate impairment of renal function. These patients are often relatively asymptomatic and, therefore, may lack sufficient motivation to adhere to this difficult regimen. For these reasons, even if protein restriction is conclusively shown to be effective in preserving renal function, it may not be applicable to many patients at risk for progressive kidney failure.

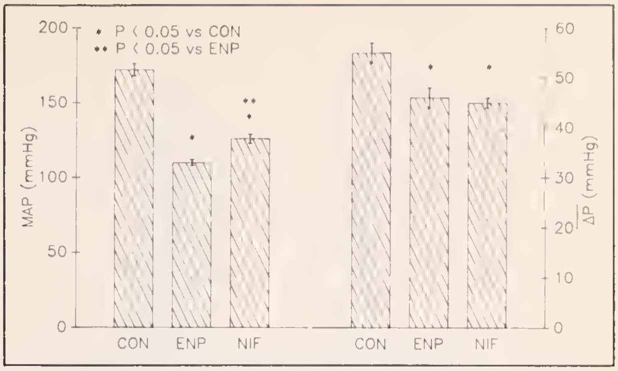


Fig. 5. Mean arterial pressure (MAP) and glomerular transcapillary hydraulic pressure difference (ΔP) in treated and untreated spontaneously hypertensive rats (SHR). CON, untreated SHR who have undergone unilateral nephrectomy; ENP, SHR treated with enalparil; NIF, SHR treated with nifedipine.

Antihypertensive Agents. One potential alternative to protein restriction to reduce the incidence of end-stage renal disease is the administration of some of the newer blood-pressure-lowering agents that have recently become available. The effect of antihypertensive therapy on glomerular hemodynamics and injury is illustrated by a study my colleagues and I performed in uninephrectomized SHR (17). An untreated group that had severe hypertension was compared to groups treated with either an ACE inhibitor or a calcium antagonist. These drugs reduced both systemic and glomerular capillary pressure (Fig. 5). In fact, the magnitude of decline in glomerular capillary pressure with drug therapy was similar to that produced by protein restriction. Associated with the decline in glomerular pressure in treated rats was a reduction in proteinuria and preservation of glomerular structure.

Thus, antihypertensive drugs may provide an alternative means to preserve kidney function. These findings have not been confirmed in humans. However, clinical trials are in progress to evaluate the efficacy of antihypertensive therapy in preserving renal function in patients with renal disease.

Renal Growth and Progressive Kidney Damage

In addition to effects on hemodynamics, both protein restriction and a variety of antihypertensive agents can inhibit kidney growth. In uninephrectomized SHR treated with enalapril or ni-

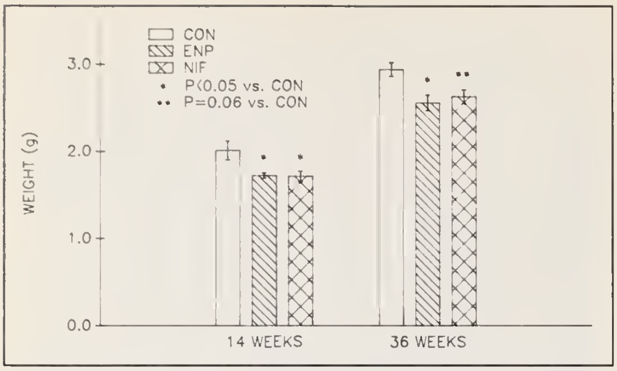


Fig. 6. Kidney weights at 14 and 36 weeks of age in treated and untreated SHR. Groups are as defined in Fig. 5.

fedipine, kidney weight was lower in treated animals than in untreated controls at both 14 and 36 weeks (Fig. 6). The effect of protein restriction on inhibiting renal growth is even more dramatic than on hemodynamics. In fact, had kidney weight been the only parameter measured, many of the animal studies I have described could have been interpreted to indicate that renal hypertrophy was the major determinant of injury and that maneuvers which lessened growth prevented glomerular sclerosis.

In most experimental settings, the hemodynamic and structural adaptations to injury occur simultaneously. However, dietary salt restriction provides a means to dissociate the hypertrophic and hyperperfusion responses and, thereby, to assess their independent roles in promoting glomerular sclerosis. We (18) compared uninephrectomized SHR fed a standard diet to animals fed a diet moderately low in salt. The degree of salt restriction selected was consistent with normal growth and development. A third group of animals received a thiazide diuretic. It had previously been demonstrated (19) that hypertension in the SHR is not salt sensitive, and we found that neither salt restriction nor the thiazide reduced systemic blood pressure in this model. There was, however, a rather dramatic effect on glomerular disease. Thus, whereas the diuretic had no effect on injury, proteinuria and glomerular sclerosis were significantly ameliorated by the low-salt diet. Our initial thought was that this protective effect would be hemodynamically mediated; however, that was not the case. Glomerular pressure was high in the untreated animals, and was similarly elevated in both the low-salt group and the thiazide-treated group. When we assessed renal growth, a different pattern was found. Salt restriction was effective in limiting compensatory kidney growth in this model. Thus, in salt-restricted rats, sclerosis was reduced when growth was inhibited, despite persistent elevation in glomerular pressure.

The Wall Tension Hypothesis. Is there a unifying hypothesis that can reconcile these apparently conflicting results and explain the adverse effects of increases in perfusion and growth on glomerular structure? One hypothesis (Fig. 7) requires that we consider the hydraulic stress to the glomerular capillary to depend on the tension which develops in the vessel wall rather than on the transmural pressure alone. Wall tension in any blood vessel is equally dependent on the pressure applied to the vessel wall and the radius of the vessel. In a setting where hypertension and hypertrophy coexist, increases in both the pressure and the radius of the vessel would tend to increase the tension in the capillary wall. Conversely, maneuvers which reduce either glomerular capillary pressure or radius will cause proportionally similar declines in tension and injury.

To determine whether glomerular capillary radius actually increases when kidneys undergo hypertrophy, we (20) studied rats with remnant kidneys (RK). This classic model of progressive renal disease is produced by surgically removing one kidney and then infarcting two thirds of remaining kidney, leaving the rat with about one sixth of the normal renal mass. Despite the fact that rats so treated have no intrinsic renal disease, the remnant tissue does not remain normal. Over several months, RK rats develop extensive glomerular sclerosis, tubular dilatation, atrophy, and cast formation (21).

In this model, it is presumed that the remnant kidney tissue is destroyed by the various adaptive processes that I have previously discussed. For example, as in the uninephrectomized SHR, glomerular injury in RK rats has been related to increases in glomerular capillary pressure and flow. Extreme reduction in renal mass also provides one of the most marked stimuli for kidney growth that can be produced. In a recent study (20), we examined the effects of salt restriction on that parameter. Animals were subjected to 1⅔ nephrectomy and were then randomized to receive either normal or low-sodium chow. A third group were fed the low-salt diet and, in addition, received repeated injections of an androgen. These hormones are potent stimulators of kidney growth but have little or no effect on glomerular hemodynamics. Our intent was to reintroduce the hypertrophy into the salt-restricted group and to see what effect this would have on injury.

As predicted, marked enlargement and a significant increase in glomerular capillary radius was observed in remnant glomeruli of rats fed normal chow but not in the salt-restricted group. Injury was severe in the control group and markedly reduced in rats on the low-salt diet. Importantly, the growth-retarding effect of salt restriction was not observed in the androgen-treated group, and the protective effect of the diet was significantly abrogated in these rats. These data suggest that the beneficial effect of salt restriction in this model depends, at least in part, on its ability to inhibit compensatory kidney growth.

Role of Growth Factors. The wall tension hypothesis is only one possible explanation for the association between stimulation of renal growth and glomerular sclerosis. An alternative possibility depends on the cellular events related to renal growth (Fig. 8). According to this scheme, an initial loss of renal function leads to the elaboration of peptide growth factors. Glomeruli mesangial cells in culture have receptors for several growth factors (22–24) and respond to them by proliferating and increasing their production of matrix material (25). It has also been demonstrated that glomerular mesangial cells release additional growth factors when stimulated (22, 23) and, therefore, can promote their own growth. Thus, a partial loss of kidney function might result in ongoing stimulation of mesangial cells to proliferate and overproduce matrix, leading ultimately to glomerular sclerosis.

Working in my laboratory in collaboration with Veronica Catanese, Judith Benstein (26) has provided preliminary evidence for increased production of at least one growth factor following surgical reduction in renal mass. She examined the changes in the renal expression of the gene which codes for insulin-like growth factor I in SHR subjected to unilateral nephrectomy. This peptide was formerly known as somatomedin C and is known to mediate many of the effects of growth hormone (GH). Dr. Benstein showed that GH-independent, kidney-specific up-regulation of IGF-I gene expression occurred after nephrectomy and suggested that this might be relevant to organ hypertrophy. Of note, other investigators have provided evidence that tissue levels of IGF-I are also increased after nephrectomy. Nevertheless, it remains to be demonstrated that increased production of this or any other growth factor is of pathophysiologic importance in promoting renal injury.

Diabetic Nephropathy

The factors I have been discussing are related to another common disease with adverse effects on the kidney, diabetes mellitus. Approximately one third of patients with Type 1 diabetes mellitus are at risk for developing diabetic nephropathy culminating in end-stage renal failure. Values for GFR reported by Mogensen (27) in normal individuals average 111 ± 13 mL/min, whereas Type 1 diabetics near the onset of their disease had elevated values averaging 156 ± 25 mL/min, demonstrating that some patients experienced a marked increase early in the course of their disease. What was not appreciated in this original report is that diabetic patients seemed to fall into two groups, those with relatively normal values and those with GFRs considerably in excess of the mean value for the group. If, as is the case after reduction in renal mass, the increase in GFR in diabetics is related to development of nephropathy, one would predict that those individuals with the highest GFRs would be at increased risk for nephropathy. In fact, when Mogensen (28) reexamined these patients 10 years later, he found that those patients who had had the highest GFRs had developed evidence of overt renal disease.

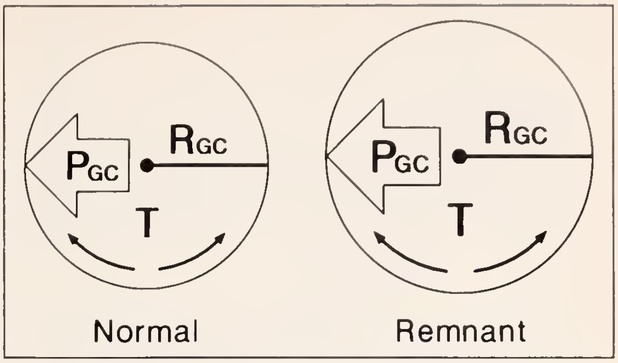


Fig. 7. Wall tension hypothesis. Tension is equally dependent on glomerular pressure (P_{GC}) and capillary radius (R_{GC}). Both parameters increase in remnant kidney and other models of progressive glomerular sclerosis. *Adapted* from ref. 33 with permission.

A similar association between alterations in glomerular function and sclerosis has been observed in rats with experimental diabetes. Zatz and coworkers (29) studied rats with diabetes mellitus induced by injection of streptozoticin. These rats also received insulin so as to maintain a moderate level of hyperglycemia. Additionally, the rats were randomized to three different levels of protein intake: 6% protein intake, 12% protein intake (low-intake groups), and 50% protein intake (high-intake group). Although all three groups of animals had similar levels of blood glucose control, the incidence of renal disease in the groups differed dramatically. In the two low-protein groups there was very little evidence for di-

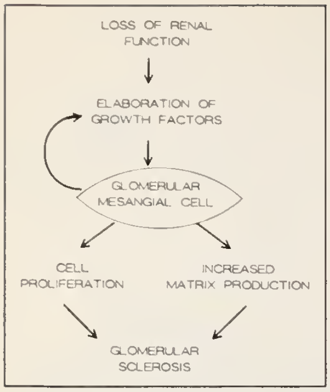

LOSS OF RENAL FUNCTION
ELABORATION OF GROWTH FACTORS
GLOMERULAR MESANGIAL CELL
CELL PROLIFERATION
INCREASED MATRIX PRODUCTION
GLOMERULAR SCLEROSIS

Fig. 8. Hypothesis relating release of growth factors to progressive glomerular damage.

abetic renal disease. In contrast, in the group that was fed the high-protein diet, marked proteinuria and glomerular sclerosis developed. When Zatz and his colleagues examined the effects of these manipulations on glomerular hemodynamics, they found that hyperfiltration and glomerular hypertension only developed in the group fed the high-protein diet.

Thus, in this model, the appearance of diabetic renal disease correlated specifically with the hemodynamic alterations associated with the diabetic state and not with the level of the blood sugar. A similar prevention of nephropathy was subsequently observed when glomerular pressure was reduced, not by protein restriction, but by the administration of antihypertensive drugs (30). These studies provide convincing evidence that experimental diabetic renal disease can be prevented by reducing glomerular pressure.

Consistent data has also been obtained in patients with type 1 diabetes in studies in which the impact of antihypertensive therapy on the course of diabetic renal disease has been examined. In the majority of these studies (31), strict control of blood pressure has resulted in a reduction in the rate of decline in GFR in patients with overt diabetic nephropathy. Beneficial effects of antihypertensive therapy have even been observed in diabetic patients without hypertension (32). Therefore, the same therapeutic maneuvers which may be effective in retarding renal damage in patients with primary renal disease may be applicable to patients with diabetes.

I would like to acknowledge the important contributions to the data I have presented of my coworkers and collaborators, including Judith Benstein, Douglas Lax, and Veronica Catanese, as well as Joseph Randazzo, Miriam Parker, and Evelyn Tolbert. Thank you.

Questions and Answers

Question: Do maneuvers such as salt restriction or antihypertensive therapy that inhibit kidney growth alter the renal expression of growth factors? What role does the renin-angiotensin system play?

Dworkin: This is something that we're very interested in and we are presently examining the effects of salt restriction on IGF1 gene expression. As yet, we haven't produced sufficient data to draw a conclusion. Nevertheless, the renin-angiotensin system doesn't seem to be the primary actor here. One piece of evidence consistent with that view is that renin levels were similarly elevated in the salt-restricted and thiazide-treated groups in the SHR study that I described. Despite the fact that both of those animals have very high renins and presumably AII levels, there were dramatic differences between the groups with respect to both kidney growth and injury. It is also possible that salt restriction may be acting by some other mechanism. In fact, the mechanism by which salt restriction alters kidney growth isn't well understood.

Richard Stein: You pointed to the agenesis of the kidney as predisposing to development of sclerosis in the other kidney; on the other hand, a fair number of studies have looked at patients who have had a kidney removed, whether for a childhood tumor, for transplant donation, or for some other reason, who have a normal remaining kidney. Although such patients have a somewhat increased incidence of hypertension and an increased incidence of a higher level of proteinuria, I'm not familiar with any studies suggesting such patients have gone on to develop significant renal failure. Would you comment on that?

Dworkin: That's correct. It may be that in humans, removing one kidney does not create a significant risk of developing end-stage renal disease. In transplant donors followed up for more than 10 years, some have observed an increase in the incidence of hypertension and some increase in protein excretion, although not to clinically significant levels. Others have found even less adverse effects. The only reservation I have with these studies is that most of the patients who have been described have not been followed for more than 10 to 15 years. Remember that renal transplantation did not become common in this country until approximately 20 years ago. In rats, whose average life span is 2 years, clinically important renal disease takes anywhere from 6 to 8 months to develop after removal of one kidney. In humans, that might translate into 30 or 40 years.

Therefore, clinically important renal disease may ultimately develop in some transplant donors. Alternatively, in humans removing one kidney may not be enough of a reduction in renal mass to stimulate these processes to the same degree that they are stimulated in rats.

Levitt: Changes in the protein content of the diet might also affect salt intake. Could the protective effect of protein restriction result from a decrease in dietary salt?

Dworkin: Salt intake is highly controlled in the low-protein diets. We add salt back to the diet so that the intake of sodium is not reduced.

Question: What about growth factors?

Dworkin: It depends on which growth factor you want to look at. Growth factors may have significant hemodynamic effects. For example, IGF-I probably mediates the increase in renal plasma flow and GFR that are seen in response to infusions of growth hormone. In fact, when infused directly into human beings or into animals, IGF-I causes a marked increase in renal plasma flow and GFR. It might be that there's a link between the hemodynamic and growth aspects of the response to a decline in renal function, and growth factors may be involved in both of these events. However, it has not been demonstrated that increased local production of IGF-I has important hemodynamic effects, and that is what is occuring in this particular system.

Question: Would you comment further on the study of Rostand that examined the incidence of renal injury in hypertensive patients?

Dworkin: I would be careful not to overinterpret that study. Despite the fact that, apparently, there were similar degrees of blood pressure control among the patients, there was a difference in the number of missed office visits. This suggests that the degree of blood pressure control may not have been precisely identical.

However, there is another possible explanation. It has been suggested that all antihypertensive agents are not equal in their ability to reduce glomerular capillary pressure and prevent injury. In some of the animal models, older drugs like hydralazine don't reduce glomerular capillary pressure, even though they control systemic hypertension. These drugs reduce systemic blood pressure yet, because they reduce preglomerular vascular resistance, glomerular pressure remains elevated and progression can occur. I don't think it's been conclusively demonstrated that particular agents are superior to others in human beings. In fact, studies in diabetics suggest that there may not be an important hierarchy among these drugs. Although different drugs have not been compared in a single study, preservation of GFR has been observed in hypertensive diabetics regardless of whether beta-blockers, hydralazine, or ACE inhibitors were used. This issue needs to be carefully investigated in humans.

Stein: Lance, thanks once again for a really elegant presentation.

References

1. US Renal Data System. *USRDS 1989 Annual Data Report.* Bethesda, Md: The National Institutes of Health, National Institute of Diabetes and Kidney Diseases, August 1989.
2. Mitch WE, Walser M, Buffingtion GA, Leman J. A simple method for estimating progression of chronic renal failure. Lancet 1976; 1326–1328.
3. Feld LG, VanLiew JB, Galeske RG, Boyland JW. Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat. Kidney Int 1977; 12:332–343.
4. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats: A consequence of glomerular capillary hypertension. J Clin Invest 1986; 77:797–809.
5. Hostetter TH, Olson JL, Rennke HG, et al. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. Am J Physiol 1981; 241:F85–F93.
6. Dworkin LD, Hostetter TH, Rennke HG, et al. Hemodynamic basis for glomerular injury in rats with desoxycorticosterone-salt hypertension. J Clin Invest 1984; 73:1448–1461.
7. Hostetter TH, Troy JL, Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus. Kidney Int 1981; 19:410–415.
8. Kiprov DD, Colvin RB, McCluskey RT. Focal and segmental glomerulosclerosis and proteinuria associated with unilateral renal agenesis. Lab Invest 182; 46:275–281.
9. Kleinknecht D, Grufeld JP, Gomez PG, Moreau JF, Garcia-Torres R. Diagnostic procedures and long-term prognosis in bilateral cortical necrosis. Kidney Int 1973; 4:390–400.
10. Cotron RS. Glomerulosclerosis in reflux nephropathy. Kidney Int 1982; 20:528–534.
11. Rostand SG, Brown G, Kirk KA, Rutsky EA, Dustan HP. Renal insufficiency in treated essential hypertension. N Engl J Med 1989; 320:684–688.
12. Rowe JW, Andres R, Tobin JD, Norris AH, Shock NW. The effect of age on creatinine clearance in man: a cross sectional and longitudinal study. J Gerontol 1976; 31:155–163.
13. Kaplan C, Pasternack B, Shah H, Gallo G. Age-related incidence of sclerotic glomeruli in human kidneys. Am J Pathol 1975; 80:227–234.
14. Anderson S, Brenner BM. Effects of aging on the renal glomerulus. Am J Med 1986; 80:435–442.
15. Bosch JP, Saccaggi A, Lauer A, Ronco C, Belledonne M, Globman S. Renal functional reserve in humans: effect of protein intake on glomerular filtration rate. Am J Med 1983; 75:943–950.
16. Ihle BU, Becker GJ, Whitwarth JA, Charlwood RA, Kincaid-Smith PS. The effect of protein restriction on the progression of renal insufficiency. N Engl J Med 1989; 321:1773–1777.

17. Dworkin LD, Feiner HD, Parker M, Tolbert E. Effects of nifedipine and enalapril on glomerular structure and function in nephrectomized SHR. Kidney Int 1991; 39:1112–1117.
18. Benstein JA, Feiner HD, Parker M, Dworkin LD. Superiority of salt restriction over diuretics in reducing renal hypertrophy and injury in uninephrectomized SHR. Am J Physiol 1990; F1675–F1681.
19. Dietz R, Schomig A, Haebara H, Mann FE, Rascher W, Luth JB, Grunherz N, Gross E. Studies on the pathogenesis of spontaneous hypertension of rats. Circ Res 1978; 43(Suppl 1):98–106.
20. Lax DS, Benstein JA, Tolbert E, Dworkin LD. Dietary salt restriction decreases renal injury in rats with remnant kidney: role of glomerular hypertropy. Am Soc Nephrol 1989; 22:323A.
21. Chanutin A, Ferris EB Jr. Experimental renal insufficiency produced by partial nephrectomy: 1. control diet. Arch Intern Med 1932; 49:767–787.
22. Lovett DH, Larsen A. Cell cycle-dependent interleukin 1 gene expression by cultured glomerular mesangial cells. J Clin Invest 1988; 82:115–122.
23. Abboud H, Peptic E, DiCorleto P. Production of platelet-derived growth factorlike protein by rat mesangial cells in culture. J Clin Invest 1987; 80:675–683.
24. Tsivitse P, Abboud HE, Saunders C, et al. Effect of epidermal growth factor on cultured mesangial cells (Abstr). Kidney Int 1987; 31:184.
25. Sterzel RB, Lovett DH, Foellmer HG, et al. Mesangial cell hillocks: nodular foci of exaggerated growth of cells and matrix in prolonged culture. Am J Pathol 1986; 125:130–140.
26. Benstein JA, Koretz K, Dworkin LD, Catanese VM. Renal insulin-like growth factor-1 gene expression is increased after unilateral nephrectomy: relationship to glomerular injury. Clin Res 1990; 38:327A (Abstr).
27. Mogensen CE. Kidney function and glomerular permeability to macromolecules in early juvenile diabetes. Scand J Clin Lab Invest 1971;28:79–90.
28. Mogensen CE, Christensen CK. Predicting diabetic nephropathy in insulin dependent patients. N Engl J Med 1984; 311:89–93.
29. Zatz R, Meyer TW, Rennke HG, Brenner BM. Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. Proc Nat Acad Sci 1985; 82:5963–5967.
30. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM. Prevention of diabetic glomerulopathy by pharmacologic amelioration of glomerular capillary hypertension. J Clin Invest 1986; 77:1925–1930.
31. Parving HH, Hommel E, Smidt UM. Protection of kidney function and decrease in albumenuria by captopril in insulin dependent diabetes with nephropathy. Br Med J 1988; 297:1086–1091.
32. Marre M, Chatellier G, LeBlanc H, Guyene TT, Menard J, Passa P. Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbumenuria. Br Med J 1988; 297:1092–1095.
33. Dworkin LD, Benstein JA. Antihypertensive agents, glomerular hemodynamics and glomerular injury. In: Epstein M, Loutzenhiser R, eds. Calcium antagonists and the kidney. Philadelphia: Hanley & Belfus, 1990:155–176.
34. Bhathena DB, Julian RG, McMorrow RG, Baehler RW. Focal sclerosis of hypertrophied glomeruli in solitary functioning kidneys of humans. Am J Kid Dis 1985; 5:226–232.

Environmental Hazards and Public Health:

Lessons for the Practice of Medicine and for Public Policy

Richard P. Wedeen, M.D., and Helen E. Sheehan, Ph.D.

Occupational diseases lie outside the mainstream of clinical practice despite the demonstrated and unchallenged scientific basis for environmental medicine. Clinical manifestations of disease in individuals heavily exposed to toxic substances in the workplace often afford the first insights into the subtle adverse effects resulting from low exposure to pollutants in the environment. Since medical practice focuses on the individual patient, it is sometimes difficult for the physician to recognize the contribution of environmental toxins to the production of chronic disease. Epidemiologic studies in large populations are usually required to demonstrate an association between exposure and adverse effects. Additional support for this linkage is suggested by converging lines of evidence from biochemistry, in vitro studies, animal observations, and intensive and expensive diagnostic testing. Implementation of the public health imperatives derived from such epidemiologic findings is often left to government officials.

In 1988, the Institute of Medicine of the National Academy of Medicine deplored the virtual absence of occupational and environmental medicine in the world of practicing physicians. In a monograph entitled "The Role of the Primary Care Physician in Occupational and Environmental Medicine," the Institute urged integration of these subjects into medical school and residency education (1). Enumerating the manifold disincentives to physicians venturing into occupational medicine—low income, low prestige, conflicts of interest, legal harassment, and fragmented government responsibility—the report also noted that those hoping to revise medical curricula faced formidable obstacles, including "institutional inertia, vested interests and competition among academic units" (p. 50). The financial viability of medical schools is tied to excellence, which is defined as scientific contribution and measured in research funds. Social responsibility, public policy, teaching, and even patient care do not fuel the fires of academe. "The average primary care physician," the report concludes, "does not consider occupational or environmental medicine part of the mainstream of medicine. Occupational medicine is considered a rather arcane subspeciality" (p. 72). Preventive medicine, which includes occupational and environmental medicine, lacks a strong professional constituency.

In 1990, the American College of Physicians echoed the concerns of the Institute of Medicine (2). Urging physicians to become responsive to the public perception of environmental degradation, the College advised internists to gain competence in risk assessment and the interpretation of epidemiologic data. An accompanying paper exhorted the profession to ensure "the academic viability of those specialists [occupational health physicians] in our medical schools" (3). It would be remarkable if such pleas, unsupported by an economic driving force, could alter professional priorities. A major revision of physicians' aspirations would have to occur. But no inexorable pres-

---

Adapted from the book tentatively entitled *Toxic Circles: From Workplace to Community,* Helen Sheehan and Richard P. Wedeen, eds, to be published by Rutgers University Press in 1992.

From the VA Medical Center, East Orange, NJ, and the Departments of Medicine and Preventive Medicine, The University of Medicine and Dentistry of New Jersey—The New Jersey Medical School, Newark, NJ. Address reprint requests to Richard P. Wedeen, M.D., VA Medical Center, East Orange, NJ 07019.

sure for such change is apparent. No equivalent of the germ theory or genetic engineering is on the horizon to raise academic prestige or economic incentives for preventive medicine. Nor will National Institutes of Health peer review panels soon be persuaded that their evaluation should include the social impact of the proposal. Increasing specialization within NIH review boards may discourage interdisciplinary research. Occupational medicine remains an unwanted stepchild of preventive medicine, a status it is likely to retain until industrial pollution produces a crisis comparable to the epidemics of centuries past. The devastation produced by toxic industrial wastes in eastern Europe and Mexico may yet prove to be crucibles for change. The promise of the Occupational Safety and Health Administration (OSHA) created in the United States in 1970 remains unfulfilled. In the 1980s, OSHA employed only one physician, and enforcement remained a political and legal game.

"Conservation" in American History. Protecting the earth from the ravages of development is less controversial than protecting people from toxic wastes. "Earth Day" now has broad appeal. But in the nineteenth century, gold, silver, copper, lead, and zinc were gouged from the American west with little concern for posterity. For decades, the shortsightedness of unbridled commercialism disturbed few (4). In the race to strike it rich, the profligate waste, the squandering of the seemingly limitless land, went virtually unnoticed. The cumulative effects of deforestation and the decimation of river beds were largely invisible to America's pioneers.

Conservation meant maximizing profits by increasing the efficiency of mineral extraction. The consequences downstream, and to the future, seemed irrelevant. To politicians, the new bacteriology fostered inventive interpretations of chemical pollution. According to the traditional theory of miasma as the cause of contagious disease, strong inorganic acids were believed to prevent the spread of infection. Some imagined that industrial effluents would cleanse the environment by killing harmful bacteria. In 1894, confronting the pollution of streams by the hatting industry, the Board of Health of West Orange Township, New Jersey, envisaged a sanitizing effect from industrial wastes in a township that lacked a sewer system:

> The factories discharge a large amount of sulfuric acid, sulphate of copper, sulphate of iron and other disinfectants into the brooks, which greatly improves the streams (5, p. 159).

Assaults on the land during the California gold rush of 1849 and the Pike's Peak rush of 1859 were slow to elicit public reaction. By the last decade of the century, however, the impact of smelters on health became a source of anxiety in Butte, Montana. The smelter owners' contention that the fumes belching from the smokestacks purified the air by killing bacteria received little credence from the town's irate citizens (4). This dispute has the ring of modern environmental controversies; the smelter smoke was unacceptable to the citizens of Butte despite the profits and wages it represented.

The price of exploitation of the land began to be perceived toward the end of the nineteenth century. Protecting wilderness gained respectability; the mountains of the west won eloquent spokesmen. As visual pollution became inescapable, John Muir raised his voice to protect the scenery. He protested the loss of wilderness to posterity. As for sickness arising from work, present and future generations were left to their own devices. No voice was raised to protect miners from pneumoconiosis. Preserving the landscape became fashionable, but the promise of profit outweighed the fear of chemical pollution. The connection between occupational disease and environmental hazards remained remote. Activists were more comfortable righting planetary ills than human afflictions. Only in the last decades of the present century has government been expected to protect the land, the worker, and the community from industrial pollution with equal commitment.

The Medical Profession and Public Health Policy. The public, frightened beyond reason by traces of substances that could not have been measured only a few decades ago, looks to bureaucrats and self-proclaimed experts for guidance and information. Physicians are bypassed. Environmental toxicology is not the province of medical practice. Prevention is relegated to pediatricians and public health workers. Occupational disease is the business of employers. Neither practitioners nor academicians are willing to redirect research priorities to mundane and recalcitrant issues of pollution. The public's quest for scientific evaluation of the hazards of industrial wastes are frustrated. Diffuse anxiety grows, and expertise is co-opted by politicians. The indifference of the medical profession elicits public distrust.

Physicians are reluctant to grant public health the status and acclaim—the legitimacy—assigned to scientific endeavors. The separation between curative medicine and public policy has a long tradition. In 1876, when Britain's chief medical officer, the influential John Simon, proposed adding public health and hygiene to medical school curricula, the newly empowered General Medical Council thwarted him (6). The Council, responsible for public health and sanitation in Victorian England, exerted a powerful influence on medical education from 1858 until the turn of the century. In the crowded urban centers of Britain, community hygiene was seen as the business of government and the sanitary police, not of physicians. The working poor could not pay the fees expected for a successful medical practice, and the unemployed sick were beyond medicine's responsibility.

The Protestant ethic had little tolerance for failure. Sickness among the destitute was widely attributed to dissolute behavior; victim-blaming marked the response to injury and disease in the workplace. The able-bodied poor were deemed unworthy because they exhibited "too little self help and too much immorality" (6, p. 396). Although holding paupers responsible for their own behavior did not necessarily exclude correcting contributing social factors, in practice those unwilling or unable to help themselves were often perceived as unworthy of the effort required for social reform.

Holding the victim responsible for injuries sustained in the workplace was usual in Victorian England. But Edwin Chadwick, the leader of poor-law reform, tempered contempt for the uncleanly habits of the working class with an awareness of socioeconomic realities. In his seminal "Report on the Sanitary Condition of the Labouring Population of Great Britain" of 1842, Chadwick noted the social consequences of industrial injuries (7):

> the prevalent impression upon such instances would be expressed by such phrases as, "if men will be so careless, there is no help for it; they must take the consequences": but they only take part of the consequences—the sickness; the main part of the consequences are taken by others, especially if they are married, when the premature widowhood and orphanage are sustained by the wife and children, who are maintained at the expense of relations or the public. The recklessness is however the result of neglected education, of which the workmen are the victims, and for measures of beneficence such workmen are regarded and to be treated as children, for they are children in intellect. (p. 320)

Despite the patronizing tone of his argument, Chadwick used this logic to justify thrusting responsibility for a clean workplace on the employer.

Sanitary Reform Movement in U.S. The sanitary reform movement in Britain was slow to find proponents in the United States. In 1837, Benjamin W. McCready, M.D., published an obscure paper, "On the Influence of Trades, Professions, and Occupations in the United States in the Production of Disease" (8). The monograph followed closely the work on diseases of occupations by John Turner Thackrah, published in Britain in 1831. McCready was concerned that many Americans "are subjected to the deleterious influence of dust or effluvia, caused by the nature of their employment; and to these evils are added others occasioned by ignorance, negligence, or custom" (8, p. 33). He urged the use of good ventilation to reduce tuberculosis and dust exposure, and provided a detailed description of industrial lead poisoning that preceded the much acclaimed monograph on lead by Tanquerel des Planches published in France in 1838 (9).

In an 1845 paper, "The Sanitary Condition of the Laboring Population of New York," John H. Griscom, M.D., City Inspector and Attending Physician at New York Hospital, shared Chadwick's view of the dependence of morals and productivity on the good health of the working class (10). "When individuals of the pauper class are ill," Griscom noted, "their entire support, and perchance that of the whole family, falls upon the community." (10, p. 1) Griscom saw a need to control epidemic infections, if not toxic and traumatic hazards in the workplace. "Ventilate, ventilate" he urged, in the hope of diminishing exposure to noxious dusts and contagious diseases among factory workers. But Griscom did not forget the need for sewage systems, temperance, and cleanliness to protect the health and morals of the poor crowded into New York's tenements (10, p. 56). His concern for the urban poor reflected his Quaker upbringing rather than the attitudes of his fellow physicians (11).

Griscom believed that the conflict between health and wealth could be resolved by "a medical education in an Officer of Health" (10, p. 50). "On the one hand, the public health is to be protected at whatever cost, on the other, private property must not unnecessarily be destroyed," he cautioned, and then asked rhetorically,

> Can it be supposed that a decision of this frequently intricate question by an officer who has little or no knowledge of chemistry, nor physiology, nor the laws of miasma, nor any other science bearing on it, will be satisfactory to the public, or to the holder of the property destroyed by his order? (10, p. 50)

His plea for physician involvement in public health policy went unheeded, a fate that can be anticipated for the recommendations of the Institute of Medicine and the American College of Physicians concerning occupational medicine in the 1990s. The sharing of income and professional rewards of curative medicine by preventive medicine remains a goal for the future.

Medical indifference to the social context of disease left the impoverished to shoulder their own misfortunes. In Victorian England, doctors were not alone in their ambivalence toward charity cases. "As the big workhouses were built and rebuilt," B. F. Smith notes of this period, "they increasingly became unknown to the outside world, while the outside world of novelists, journalists and social critics was content not to know" (6, p. 382). According to the medical model of "cases," the healthy unemployed were the unworthy poor; not people in need of help, but misfits and deviants in need of discipline. Compassion was tempered by the potential for "pauperization": the perpetuation of sloth, immorality, and poverty as a consequence of help received.

American attitudes toward the working poor derived from European traditions. In the voluntary hospitals of the antebellum South, when the ingratitude of indigent patients led to confrontation with hospital benefactors, offending patients were punished with jail, reduced rations, or cold showers (12). In return for benevolence, the indigent were expected to conform to societal norms. Recipients of public largesse were expected to exhibit gratitude, conformity, and thrift (6). Pecuniary care was justified as deterrence; humiliation modulated unrestrained charity, averting pauperization. The worthy poor, reduced to dependence by illness or old age, were largely ignored. The infirm, widowed, senile, mentally incompetent, insane, deaf, blind, and dying were sequestered, preferably out of sight, in alien institutions.

"Pauperism is a social evil and a sign of moral weakness" stated the New Jersey Bureau of Statistics of Labor and Industries in the Annual Report of 1883 (13, p. 373). "The suppression of pauperism can be accomplished only by preventing what are held to be its chief causes: the neglect of destitute and pauper children, the improper administration of our alms-houses, and indiscriminate charity" (13, p. 418). While reflecting a conventional view of poverty, the Bureau also recognized a connection between wages and pauperism. "Insufficient compensation for labor brings on poverty," the report noted with nonjudgmental clarity (13, p. 419). Nevertheless, the Essex County poorhouses estimated that "Ninety per cent. of the pauperism was caused by intoxicants; ten per cent. is the result of the infirmities of old age" (13, p. 385). Government provision of social support began later in the United States than in Britain in part because of the dominant role of private assistance organizations, and a deep, not unwarranted suspicion of political patronage and corruption in the United States (14).

The separation of occupational medicine from the mainstream of medical practice thus has strong economic and cultural roots. The pioneer contributions of Alice Hamilton at the turn of the century initially found few followers in the field of occupational medicine. Physician involvement did not become more than token until the advent of state worker's compensation laws between 1908 and 1911. These laws were created at the behest of industry to stem the tide of expensive court decisions. They provided payment for medical expenses for traumatic injuries for those who could prove they had been injured on the job (15). Compensation for chronic diseases contracted in the workplace remained largely beyond the purview of compensation law because etiology was uncertain. Koch's postulates could not be met by mesotheliomas appearing three decades after exposure to asbestos. Although in theory, silicosis was often covered by worker's compensation law, in practice proving a single cause was extremely difficult until the x-ray findings were unequivocal or postmortem specimens were in hand. Asbestosis and silicosis came under New Jersey's worker's compensation statutes in 1944, but the issue is still being contested in the courts. The pneumoconioses effectively remained uncompensable until covered by federal statutes in 1970. The long-delayed appearance and multifactorial nature of chronic disease rendered etiology problematic. Industry capitalized on this uncertainty by perennially supporting more research rather than prevention in the workplace. The medical investigator's insatiable quest for research funds found a natural alliance with industry's demand for etiologic certainty: Decisions on worker protection can be deferred indefinitely under the guise of seeking scientific truth.

As a result of the states' compensation laws, industry found a need to hire physicians to defend against false claims (16). Company physicians were essential to perform pre-employment physicals and identify workers with prior illness or handicaps. Although these efforts might benefit the workers, the obvious divided loyalties of company physicians created a climate of distrust for occupational medicine that has yet to be dissipated.

Summary

The separation of occupational and environmental disease from the mainstream of medical practice has deep roots in the culture of the profession. Medical practice centered on individual patient care as nineteenth-century science yielded the therapeutic triumphs of the twentieth century. Social issues seemed remote to medical practitioners as the rewards of scientifically based therapies upstaged the unglamorous aspects of preventive medicine. Public health was left to politicians and bureaucrats. Victorian ambivalence toward the less successful members of society reinforced the isolation of medicine from public policy. As a consequence, physicians are largely ignored in contemporary debates about environmental hazards, to the detriment of both society and the profession.

References

1. Warren J, ed. The role of the primary care physician in occupational and environmental medicine. Washington, D.C.: The Institute of Medicine, the National Academy of Medicine, 1988.
2. American College of Physicians. Position paper. Occupational and environmental medicine: the internist's role. Ann Int Med 1990; 113:974–982.
3. Castorina JS, Rosenstock L. Physician shortage in occupational and environmental medicine. Ann Int Med 1990; 113:983–986.
4. Smith DA. Mining America: the industry and the environment, 1800–1980. Lawrence: University Press of Kansas, 1987, 21.
5. Board of Health of the State of New Jersey, 18th Annual Report, 1894; Doc. No. 5, p. 159, in Legislative Documents of the State of New Jersey, Vol. I, Documents 1 to 13, inclusive, pub. in 1895.
6. Smith FB. The people's heath 1830–1910. New York: Holmes and Meier, 1979.
7. Chadwick E. Report on the sanitary condition of the labouring population of Gt. Britain. 1842. M. W. Flynn, ed. Edinburgh: Edinburgh University Press, 1965.
8. McCready BW. On the influence of trades, professions, and occupations in the United States, in the production of disease. (1837). Reprint of 1943 edition. New York: Arno Press and the New York Times, 1972.
9. Wedeen RP. Poison in the pot: the legacy of lead. Carbondale, IL: Southern Illinois University Press, 1984.
10. Griscom JH. The sanitary condition of the laboring population of New York. With suggestions for its improvement. A discourse (with additions) delivered on the 30th December, 1844, at the Repository of the American Institute. New York: Harper & Brothers, 1845.
11. Rosenberg CE, Smith-Rosenberg C. Pietism and the origins of the American public health movement: a note on John H. Griscom and Robert M. Hartley, In: Leavitt JW, Numbers RL, eds. Sickness and health in America. Madison: The University of Wisconsin Press, 1978, 345–349.
12. Rosenberg CE. The care of strangers: the rise of America's hospital system. New York: Basic Books, 1987.
13. Bureau of Statistics of Labor and Industries of New Jersey, Sixth Annual Report, Year ending Oct. 31, 1883. Part VI. Pauperism. Documents of the One Hundredth and Eighth Legislative Session of the State of New Jersey, Vol I, Documents No. 1–14, 1884, 371–444.
14. Orloff AS, Skopcol T. Why not equal protection? Explaining the politics of public social spending in Britan, 1900–1911, and the United States, 1880–1920. Am Sociolog Rev 1984; 49:726–750.
15. Gelman JL. New Jersey practice. Volume 38, Worker's compensation law. St. Paul, Minn.: West Publishing, 1988.
16. Sellers C. The Public Health Service's office of industrial hygiene and the transformation of industrial medicine. Bull Hist Med 1991; 65:42–73.

Diminished Ratio of Estrogen Receptors to Progesterone Receptors in Breast Carcinomas of Women Who Have Had Multiple Miscarriages

Steven Lehrer, M.D., Eva Levine, M.S., Penny Savoretti, R.N., Joan Cropley, R.N., Jack Dalton, M.D., H. K. Song, M.D., Swan N. Thung, M.D., Jack Rabinowitz, M.D., and Lynda Mandell, M.D.

Abstract

We examined the relationship of a history of miscarriage to the ratio of tumor estrogen receptors to progesterone receptors in 74 women with breast carcinoma evaluated between 1988 and 1990. Only women who had been pregnant and whose tumor contained both estrogen and progesterone receptors were included. Women with breast carcinoma and a history of two to four miscarriages had significantly lower ratios (<1) of tumor estrogen receptors to progesterone receptors than women with a history of zero or one miscarriage (>1). This finding may be the result of a genetic anomaly in breast cancer patients that also affects the uterine environment during the reproductive years.

Many studies have shown that risk factors for the development of breast carcinoma affect tumor estrogen receptor (ER) levels. Among these factors are late age at first full-term pregnancy (1), history of benign breast disease (2), nulliparity (3), history of breast feeding (3), race (4), age at diagnosis (4–6), history of oophorectomy (7), use of oral contraceptives (7, 8), and body weight (9).

Pike et al. (10) and Hadjimichael et al. (11) have shown that abortion, whether spontaneous (miscarriage) or induced, poses a risk for the development of breast carcinoma. In addition, Brinton et al. (12) demonstrated that multiple miscarriages were also a risk factor.

In this study, we examined the relationship of a history of miscarriage to the ratio of tumor estrogen receptors to progesterone receptors in women with breast carcinoma.

Materials and Methods

All the women in this study had been referred for therapy of a primary breast carcinoma. A registered nurse administered a questionnaire to the patients to obtain historical and clinical data. ER and progesterone receptor (PR) levels were determined with a commercially available dextran-coated charcoal assay kit (Rianen Assay System, Dupont Medical Products, N. Billerica, MA). The kit works by titrating binding sites in the tumor cytosol with increasing concentrations of tritiated estradiol or R5020, a sythetic progestin. Separation of the tightly bound receptor-hormone complexes from unbound steroid is achieved by adsorption of the free material onto dextran-coated charcoal. After centrifugation, the supernatant is decanted and counted. Scatchard plot analysis of the specific binding data is used to determine binding capacity and affinity.

The 74 women in this study were evaluated between 1988 and 1990. Only women who had been pregnant, and whose tumor contained both ER and PR, were included. Statistical analysis was performed with Student's *t*-test (pooled variance analysis) or Welch's t-test (separate variance analysis).

From the Department of Radiation Therapy and the Stratton-Popper Department of Pathology (SNT), Mount Sinai School of Medicine (CUNY), New York, NY. Address reprint requests to Steven Lehrer, M.D., Radiation Therapy Department, Box 1236, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

Results

Of the pregnancies in this study, 22% ended in miscarriage. There was a significantly lower tumor ER/PR ratio in breast cancer patients with a history of two to four miscarriages (p = 0.010 two-tailed; see Fig. 1). There was, however, no difference in the levels of ER or PR alone (Fig. 2). We have analyzed square root (ER/PR) to normalize the distribution. Note that in breast cancer patients with a history of zero or one miscarriage, the ratio is greater than one, whereas in patients with a history of multiple miscarriages the ratio is less than one. This dichotomy was chosen because habitual abortion is broadly defined as the loss of two or more pregnancies before fetal viability (13).

Because ER/PR ratio is correlated with age (see Fig. 3), we examined the association of age of the breast cancer patients with multiple miscarriages. No relationship was found (Fig. 4).

Discussion

Miscarriage is common during pregnancy. Mills et al. found that recognized miscarriage occurs in 16% of normal pregnancies (14). Wilcox et al. found that recognized miscarriage occurs in 12% of normal pregnancies (15). But the 22% miscarriage rate we report here in women with breast cancer is relatively high compared with the normal rate.

Multiple studies have demonstrated a need for estrogen in early pregnancy. Ravindranath and Moudgal inhibited pregnancy in bonnet monkeys by administering tamoxifen, an antiestrogen, during the postovulatory period (16). Furr et al. (17) found that if tamoxifen was orally administered to rabbits on day 10 after implantation, fetal resorption resulted. Later tamoxifen treatment caused a significant reduction in the length of gestation and number of live young born. Because estrogen is necessary in early pregnancy,

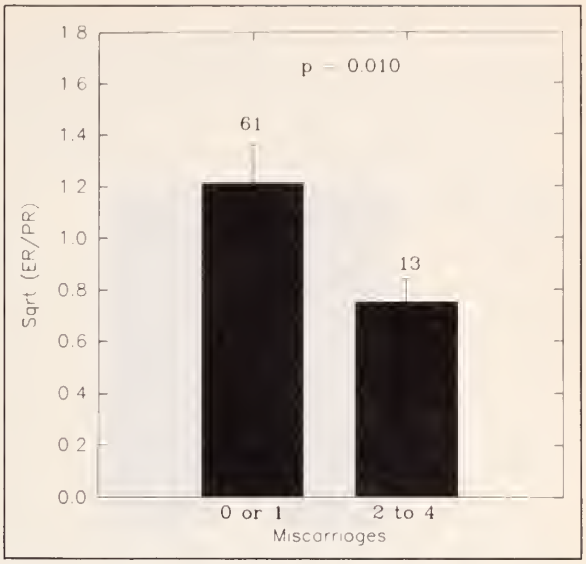


Fig. 1. Effect of miscarriage on square root of ratio of estrogen receptors to progesterone receptors (Sqrt [ER/PR]). Women with a history of 2 to 4 miscarriages had a lower Sqrt (ER/PR) than women with a history of 0 or 1 miscarriage (separate variance estimate) (mean ± SEM).

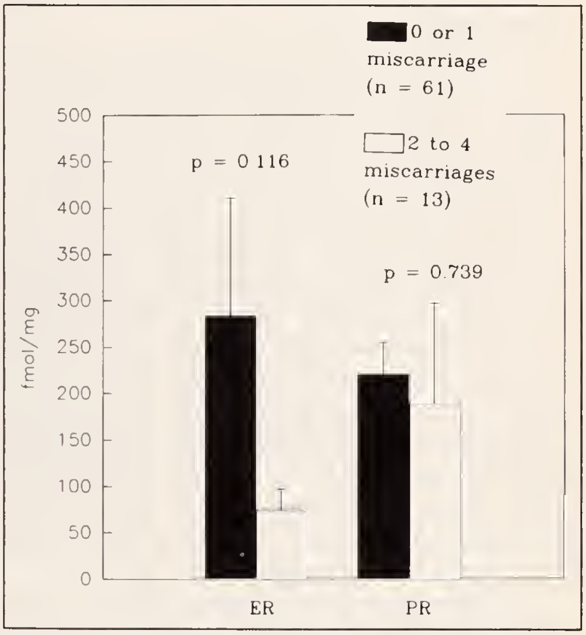


Fig. 2. Effect of history of miscarriage on ER or PR levels alone (Student's t-test) (mean ± SEM).

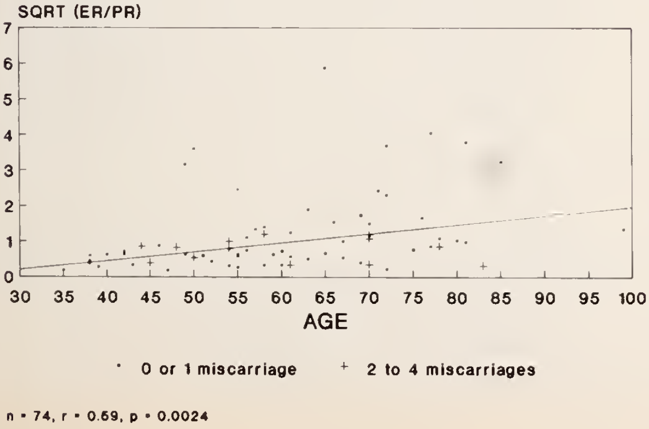


Fig. 3. Effect of age on ratio of estrogen receptors to progesterone receptors in breast cancer. Sqrt (ER/PR) measured in breast cancers increases with age.

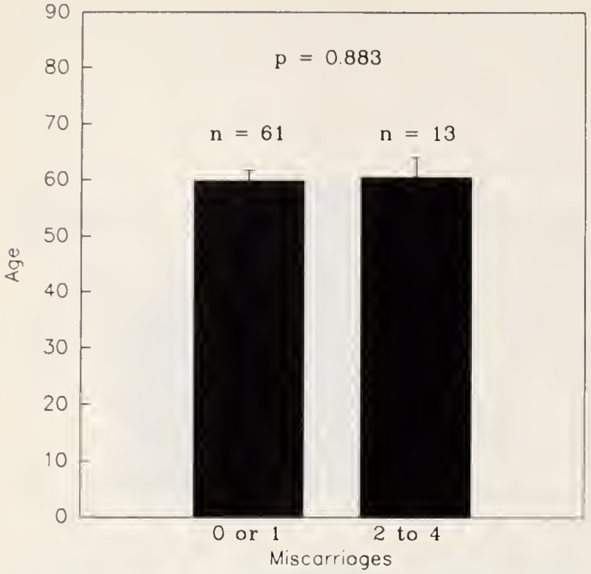


Fig. 4. Influence of history of miscarriage on age of breast cancer patients (Student's *t*-test) (mean ± SEM).

the ER, too, must play an important role in pregnancy maintenance.

There is evidence that naturally occurring variations in the structure of the ER gene can affect its function. Hill et al. (18) examined a single, two-allele restriction fragment length ER polymorphism in human breast carcinoma cell lines and human breast tumor biopsy specimens. Their studies showed an association between absence of one allele and absence of ER expression.

There is a high incidence of miscarriage in women with breast carcinoma (10–12). Moreover, our finding of diminished ER/PR ratio in the breast carcinomas of women with a history of two to four miscarriages suggests that an ER anomaly might be responsible for the diminished ER/PR ratio and the miscarriages, since the synthesis of PR is controlled by ER (19). One such anomaly has already been identified (20).

The findings in this study suggest that the ER/PR ratio in the breast tumors reflects the uterine environment. This is not surprising because the same genes control synthesis of both uterine and breast tumor ER and PR. Furthermore, our findings imply that PR level, in addition to being controlled by ER, is regulated by a feedback mechanism. Some breast cancer patients have a genetic change that resets this mechanism and diminishes the ER/PR ratio, thus predisposing to miscarriage during the reproductive years and to breast cancer later.

Acknowledgments

We thank the Edgar McCoubrey Foundation for its generous support.

References

1. McTiernan A, Thomas DB, Johnson LK, Roseman D. Risk factors for estrogen receptor–rich and estrogen receptor–poor breast cancers. J Natl Cancer Inst 1986; 77:849–854.
2. Stanford JL, Szklo M, Boring CC, et al. A case-control study of breast cancer stratified by estrogen receptor status. Am J Epidemiol 1987; 125:184–194.
3. Hildreth NG, Kelsey JL, Eisenfeld AJ, LiVolsi VA, Holford TR, Fischer DB. Differences in breast cancer risk factors according to the estrogen receptor level of the tumor. J Natl Cancer Inst 1983; 70:1027–1031.
4. Hulka BS, Chambless LE, Wilkinson WE, Deubner DC, McCarty KS Sr, McCarty KS Jr. Hormonal and personal effects on estrogen receptors in breast cancer. Am J Epidemiol 1984; 119:692–704.
5. Ballard-Barbash R, Griffin MR, Fisher LD, Covalciuc MA, Jiang N. Estrogen receptors in breast cancer. Association with epidemiologic risk factors. Am J Epidemiol 1986; 124:77–84.
6. Elwood JM, Godolphin W. Oestrogen receptors in breast tumors: associations with age, menopausal status and epidemiological and clinical features in 735 patients. Br J Cancer 1980; 42:635–644.
7. Wallace RB, Sherman BM, Bean JA. A relationship between artificial menopause, previous estrogen consumption, and estrogen receptor content of breast neoplasms. Oncology 1980; 37:111–113.
8. Lesser ML, Rosen PP, Senie RT, Duthie K, Menendez-Botet C, Schwartz MK. Estrogen and progesterone receptors in breast carcinoma: correlations with epidemiology and pathology. Cancer 1981; 48:299–309.
9. Nomura Y, Tashiro H, Hamada Y, Shigematsu T. Relationship between estrogen receptors and risk factors of breast cancer in Japanese pre- and postmenopausal patients. Breast Cancer Res Treatment 1984; 4:37–43.
10. Pike MC, Henderson BE, Casagrande JT, Rosario I, Gray GE. Oral contraceptive use and early abortion as risk factors for breast cancer in young women. Br J Cancer 1981; 43:72–76.
11. Hadjimichael OC, Boyle CA, Meigs JW. Abortion before first livebirth and risk of breast cancer. Br J Cancer 1986; 53:281–284.
12. Brinton LA, Hoover R, Fraumeni JF. Reproductive factors in the aetiology of breast cancer. Br J Cancer 1983; 47:757–762.
13. Maier DB. Genetic and infectious causes of habitual abortion. In: Gondos B, Riddick DH, eds. Pathology of infertility. New York: Thieme, 1987:201–218.
14. Mills JL, Simpson JL, Driscoll S, et al., and the National Institute of Child Health and Human Development-Diabetes in Early Pregnancy Study. Incidence of spontaneous abortion among normal women and insulin-dependent diabetic women whose pregnancies were identified within 21 days of conception. N Engl J Med 1988; 319:1617–1623.
15. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. N Engl J Med 1988; 319:189–194.
16. Ravindranath N, Moudgal NR. Use of tamoxifen, an antiestrogen, in establishing a need for estrogen in early pregnancy in the bonnet monkey (*Macaca radiata*). J Reprod Fertil 1987; 81:327–336.
17. Furr BJA, Valcaccia B, Challis JRG. The effects of Nol-

vadex (tamoxifen citrate; ICI 46,474) on pregnancy in rabbits. J Reprod Fertil 1976; 48:367–369.

18. Hill SM, Fuqua SAW, Chamness GC, Greene GL, McGuire WL. Estrogen receptor expression in human breast cancer associated with an estrogen receptor gene restriction fragment length polymorphism. Cancer Res 1989; 49:145–148.
19. Clarke CL, Feil PD, Satyaswaroop PG. Progesterone receptor regulation by 17-beta-estradiol in human endometrial carcinoma grown in nude mice. Endocrinology 1987; 121:1642–1648.
20. Lehrer S, Sanchez M, Song HK, et al. Oestrogen receptor B-polymorphism and spontaneous abortion in women with breast cancer. Lancet 1990; 335:622–624.

Submitted for publication March 1990.
Final revision submitted February 1991.

The Correlation between Lupus Anticoagulant and Autoantibodies

Norbert Gleicher, M.D., Lisa Harlow, and Albert El-Roeiy, M.D.

Abstract

The presence of a positive lupus anticoagulant (LA) has recently been associated with reproductive failure. LA positivity and reproductive failure have also been associated with autoantibody abnormalities, especially antiphospholipid autoantibodies. Because the correlation between LA and autoantibody positivity has remained controversial, we retroactively investigated 326 sera of patients with reproductive failure for correlation between lupus anticoagulant and 15 autoantibodies separately for IgG, IgM, and IgA isotypes.

LA by TTI and APTT correlated significantly ($p < 0.0001$). Among 18 antiphospholipid isotypes, 6 (33%) correlated significantly with TTI, 5 (28%) with APTT, and 3 (17%) both with TTI and APTT. Among 15 isotypes to histone subfractions, 4 (27%) correlated significantly to TTI, 3 (20%) to APTT, and 2 (13%) to both LA assay methods. Of 12 isotypes to antipolynucleotide antibodies, 2 (17%) correlated significantly to TTI, 1 (8%) to APTT, and none to both assay methods. The correlation between LA and autoantibodies was less striking among LA-positive than LA-negative sera.

LA by both TTI and APTT was primarily found to correlate with IgG and IgA autoantibodies. This occurs in decreasing frequency to phospholipids, histones, and polynucleotides. At abnormal levels of LA (by either method) the correlation with autoantibody levels is less pronounced and appears closer with IgM autoantibodies to histones and polynucleotides than antibodies to phospholipids.

We conclude that the correlation between LA and autoantibodies, especially antiphospholipid antibodies, is less pronounced than often reported in the literature. Patients suspected to suffer from reproductive failure due to abnormal autoimmunity have to be screened not only with LA and/or selected antiphospholipid autoantibodies, but with a more comprehensive autoantibody profile.

Abnormal lupus anticoagulant (LA) was initially associated with pregnancy wastage (1, 2). Because of the known association between LA and abnormal autoantibodies, especially antiphospholipid antibodies, patients with repeated pregnancy wastage and positive LA were subsequently evaluated for selected antiphospholipid antibodies (3–5). These studies led to the observation that patients with repeated pregnancy wastage and positive LA also demonstrated significant autoantibody abnormalities among antiphospholipids (3–5). One laboratory, in fact, suggested that LA correlated perfectly with the phospholipid antibody antiphosphatidylserine and that such a correlation existed only if LA was evaluated by APTT (6). Others suggested strong correlations with cardiolipin (4, 5) and phosphatidic acid antibodies (7).

LA and autoantibody abnormalities to phospholipid, histone, and polynucleotide antigens have since also been associated with endometriosis (8), unexplained infertility (9), IVF failure (10), and the presence of sperm antibodies (11). The correlation between LA and broadly based

---

Presented in part at the 8th Annual Meeting of The American Society for The Immunology of Reproduction, Portland, Maine, June 1988.

Supported in part by the Foundation for Reproductive Medicine, Inc. Chicago, Illinois 60610 and a Mount Sinai Hospital Medical Center Research Center Grant.

From the Division of Reproductive Immunology, Department of Obstetrics and Gynecology, Mount Sinai Hospital Medical Center of Chicago and Rush Medical College, Chicago, Illinois. Address reprint requests to Norbert Gleicher, M.D., Center for Human Reproduction, 750 N. Orleans St., Chicago, IL 60610.

TABLE 1

*Reported Diagnoses of Study Population**

Reported diagnosis	No.
Repeated abortions	154
Pregnancy (normal and abnormal)	136
Systemic lupus erythematosus	60
Endometriosis	56
Unexplained infertility	46
Reproductive autoimmune failure syndrome	11
Arthritis	9
Anovulation	8
Threatened abortion	8
Sperm antibodies	6
Intrauterine fetal demise	6
In vitro fertilization	6
Previously positive ANA/DNA	6
Thrombocytopenia	3
Preeclampsia	3
Unknown	3
Newborn	3
Allergy	3
Tubal disease	3
Sarcoidosis	3
Intrauterine growth retardation	2
Bell's palsy	2
Luteal phase deficiency	2
Central nervous system thrombosis	2
Hypertension	1
Multiple sclerosis	1
Chronic hepatitis	1
Colitis	1
Mixed connective tissue disease	1

* Number of diagnoses exceeds number of patients because many patients had more than one diagnosis. All sera were received from women with reproductive problems. Newborn sera came from children whose mothers had severe preeclampsia.

autoantibody abnormalities has therefore assumed importance for the diagnosis of a broad spectrum of reproductive failure. Our laboratory has served as referral center and screening laboratory for a large population of patients with various LA and autoantibody-associated forms of reproductive failure. Collection over approximately a 12-month period of 326 sera, which had been concomitantly evaluated for LA and a broadly based autoantibody screen, allowed a retroactive analysis of correlation patterns that is the basis for this report.

Material and Methods

A total of 326 sera had been concomitantly analyzed for LA and a broadly based autoantibody screen since the autoantibody laboratory of the Department of Obstetrics and Gynecology at MSHMC of Chicago had been fully computerized. All data on these 326 sera were consequently stored in computer memory and were retroactively retrievable. Table 1 summarizes the diagnoses of investigated patients.

LA was investigated as previously described in detail (8), utilizing two standard methods: The tissue thromboplastin inhibition test (TTI) was performed by the method of Proctor and Mannucci et al. (12). The APTT was determined as suggested by Rappaport (13) using a partial thromboplastin prepared from human brain by the method of Bell and Alton (14). Autoantibodies to 6 phospholipid antigens (cardiolipin, phosphatidylserine, phosphatidic acid, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol), 5 histone subfractions (H1, H2A, H2B, H3, H4), and 4 polynucleotides [ssDNA, dsDNA, poly I, poly(dT)], were separately investigated by ELISA assay for IgG, IgM, and IgA isotypes, as previously described in detail (8).

Statistical correlation between LA and autoantibody levels was established using a Pearson correlation matrix from the statistical analysis package for change by Systat on a Compac 286 computer. For the total study group ($n = 326$) the significant ($p = 0.05$) r value was 0.109, for group A ($n = 22$) $r = 0.422$, for group B ($n = 25$) $r = 0.396$, and for group C ($n = 13$) $r = 0.550$.

Results

LA, as evaluated by TTI and APTT, correlated significantly ($p < 0.0001$) in all 326 patient samples. Twelve of 45 investigated autoantibody isotypes (27%) correlated significantly to LA, as evaluated by TTI. In contrast, only 9 (20%) of antibodies correlated to APTT (Table 2). Five autoantibodies (11%) correlated significantly to both TTI and APTT. These were: IgG antiphosphatidic acid, IgA antiphosphatidylethanolamine, IgA antiphosphatidylinositol among antiphospholipids, and IgG anti-H-1 and anti-H-4 among antihistone subfractions.

Among antiphospholipids, 6 of 18 isotypes (33%) correlated with TTI and 5 (28%) with APTT. Four of 15 antihistone isotypes (27%) correlated with TTI and 3 (20%) with APTT. Two of 12 (17%) antipolynucleotid isotypes correlated significantly with TTI and only 1 (8%) with APTT.

Of 12 significantly correlating isotypes to TTI-LA, 6 were IgG and 6 IgA, while the distribution for those correlating to APTT-LA was: 5, IgG; 1, IgM; 3, IgA. The 5 isotypes that correlated to TTI as well as APTT were 3 IgG and 2 IgA isotypes.

Twenty-two of the 326 samples demonstrated a TTI greater than 1.3 (group A). Table 3 demonstrates that TTI-LA correlates to none of the 45 autoantibody isotypes in these patients except IgG anti-poly(dT) ($p = 0.003$). This isotype also correlated significantly for the total patient population ($p < 0.0001$). In contrast, 5 isotypes correlated in group A patients with APTT-LA. These included IgA antiphosphatidylinositol, IgG anti-H2A, IgM anti-H3, IgG anti-ssDNA, and IgM anti-dsDNA. Only IgA antiphosphatidylinositol was also significantly correlated for the whole group ($p < 0.0001$).

Twenty-five patients demonstrated an APTT greater than 40 sec (group B). Group B patients demonstrated significant correlations between TTI-LA and IgG as well as IgA antiphosphotidic acid and IgG anti-poly(dT). All three isotypes also correlated significantly to the whole group. APTT-LA correlated in this group with IgA antiphosphatidylinositol, IgM anti-H2A, IgM anti-H2B, and IgM anti-H3. Of those, only IgA-antiphosphatidylinositol correlated to the whole group.

Thirteen patients demonstrated a TTI of 1.3 or greater and an APTT greater than 40 sec (group C). Among those, TTI-LA correlated significantly with IgM anti-H4, IgA anti-ssDNA, and IgG anti-poly(dT). Only IgG anti-poly(dT) correlated also with the whole group. APTT-LA correlated significantly with IgM anti-H2A, -H2B, and -H3. None of those IgM antihistones also correlated with the whole study group.

TABLE 2

*Significant Correlation between LA (by TTI and APTT) and Autoantibodies**

	Lupus	Anti-coagulant†
Autoantibody	TTI	APTT
Phospholipids		
IgG anticardiolipin		0.05
IgA anticardiolipin	0.032	
IgG antiphosphatidylserine		0.01
IgG antiphosphatidic acid	0.007	0.034
IgA antiphosphatidic acid	0.006	
IgA antiphosphatidyl-ethanolamine	0.007	0.009
IgG antiphosphatidylglycerol	0.001	
IgA antiphosphatidylinositol	0.042	0.0001
Histones		
IgG anti-H1	0.0001	0.0001
IgA anti-H1	0.0009	
IgA anti-H2A		0.018
IgA anti-H2B	0.017	
IgG anti-H4	0.0001	0.0001
Polynucleotides		
IgA anti-poly I	0.0001	
IgG anti-poly(dt)		
IgM anti-poly(dt)		

* Only significant correlations with six antiphospholipids, five antihistones, and four antipolynucleotides are shown.

† Numbers indicate p-value.

Discussion

The correlation between LA positivity and autoantibody positivity has remained controversial. While it is generally accepted that a positive LA is associated with abnormal autoimmune function of B lymphocytes, the degree of this association has never before been defined for more than a limited number of arbitrarily selected autoantibodies. Moreover, correlations between LA and autoantibodies usually referred only to LA-positive patient populations and thus introduced a clear selection bias. We noted this bias in previous publications by demonstrating that the autoantibody abnormalities found in LA-positive repeated aborters varied from those of LA-negative patients with repeated pregnancy wastage (9). Cowchock et al. (4) reported similar results restricted to phospholipid antibodies and certain antinuclear antibodies. Concomitantly, we were unable to demonstrate in other autoantibody-positive patient populations (8) the close correlation between LA and autoantibody positivity that was alleged by other centers (6).

A normal LA is usually defined as a TTI below 1.2. A TTI between 1.2 and 1.3 is considered borderline and a level above 1.3 represents a positive result (12). Some investigators have suggested that the TTI is unreliable as a predictor of autoantibody positivity and have proposed instead the utilization of the APTT to detect LA (6). An APTT greater than 40 sec can be considered indicative of the presence of LA (6, 8, 12). In principle, many phospholipid-dependent coagulation tests can be utilized to detect LA (15). LA is, however, defined only by the prolongation of these tests if correction of the coagulation assay with a normal patient sample does not reverse the prolongation (15). Such a correction is routinely performed in our laboratory but has not been the universal practice elsewhere. This discrepancy in assay methodology may explain some of the difference in correlation between LA and autoantibody levels observed between our and other laboratories.

Normal patient populations express normal LA and normal autoantibody levels. We have confirmed the published normal TTI and APTT levels for our own laboratory and population and have also demonstrated that LA is not affected by the hormonal changes of the ovulatory menstrual cycle (16). Normal autoantibody levels have also

TABLE 3

*Significant Correlations between LA and Autoantibodies in Only LA-Positive Patients of Groups A, B, and C**

	Lupus anticoagulant p value					
	Group A (n = 22)		Group B (n = 25)		Group C (n = 13)	
Autoantibody	TTI	APTT	TTI	APTT	TTI	APTT
Phospholipids						
IgG antiphosphatidic acid			0.023			
IgA antiphosphatidic acid			0.028			
IgA antiphosphatidylinositol		0.018		0.002		
Histones						
IgG anti-H2A		0.009				
IgM anti-H2A				0.049		0.024
IgM anti-H2B				0.041		0.051
IgM anti-H3		0.029		0.036		0.034
IgM anti-H4					0.03	
Polynucleotides						
IgG anti-ssDNA		0.03			0.019	
IgA anti-ssDNA						
IgG anti-dsDNA		0.018				
IgG anti-poly(dt)	0.003		0.003		0.001	

* Only significant correlations with six antiphospholipids, five antihistones, and four antipolynucleotides are shown.

been rigidly defined by establishing a 99% confidence limit for each of the autoantibodies and their isotypes reported here (17). In other correlation studies, abnormal autoantibodies have been defined as standard deviations of rather small control groups (18). Such an analysis of data will result in a large number of positive samples in normal populations since phospholipid antibodies, antihistones, and some antipolynucleotides are nonparametrically distributed (7, 17). Since both LA and autoantibodies can be detected at "normal" levels, a significant correlation between the two should carry into normal levels of each of these assays. Previous studies largely ignored this fact by investigating only correlations and LA and/or autoantibody-positive patients (3, 6, 7, 18). The present study was designed to overcome such a selection bias.

This study contradicts the previously reported strong correlation between LA and autoantibodies to some phospholipid antigens (6). At the same time, this study confirms the fact that among various autoantibody groups, autoantibodies to phospholipids appear to have the closest correlation with a positive LA, followed by those to histones and, finally, those to polynucleotides. This correlation seems present independent of the method by which the presence of LA is determined. The validity of these results is further confirmed by the fact that our LA determinations by TTI and APTT correlated significantly ($p < 0.0001$).

Quite surprisingly, the correlation between LA and autoantibodies decreased even further when only LA-positive patients were investigated. This may at least partially be due to the smaller number of investigated sera in groups A, B, and C in comparison to the whole study population. LA-positive patients also lost the relative close correlation to antiphospholipid antibodies. In fact, LA-positive patients demonstrated correlations primarily with autoantibodies to histones and polynucleotides.

Another important issue that became apparent was that autoantibody isotypes, which correlated statistically with LA in the whole patient population, were almost universally IgG or IgA. In fact, of 21 isotypes that significantly correlated to either TTI or APTT, only 1 was an IgM; the remaining were evenly distributed between IgG and IgA. In contrast, among LA-positive patients (groups A, B, and C), 9 of 19 isotypes were IgM immunoglobulins. This difference is statistically highly significant ($p < 0.0007$, chi-square). IgG and IgM isotypes of (phospholipid) antibodies have been suggested to have different clinical importance. Some investigators have proposed that IgG, but not IgM, autoantibodies are associated with thrombosis (19). Others indicated a similar association with reproductive failure (5, 20). Triplett et al. (18) were, however, unable to confirm such an association. The data presented here suggest that LA positivity may indicate a disproportionate incidence of IgM isotypes in primarily antihistone and antipolynucleotide antibodies. Since most investigators have concentrated on the study of antibodies to phospholipid antigens, this observation has not been made before. It has potential diagnostic as well as clinical importance because it suggests that other than phospholipid specificities should be investigated in potentially affected individuals.

Autoantibodies, including monoclonal lupus autoantibodies, are usually polyspecific (21). Cross-reactivities and cross-specificities are characteristic of various autoantibody groups and classes (22). Antibodies to phospholipids are also known to cross-react with antihistones and antipolynucleotides. The correlation reported here between LA and autoantibodies to histones as well as polynucleotides is therefore no surprise. In women with reproductive wastage, this association was reported before (8, 9). In fact, IgM isotypes of autoantibodies to histones and polynucleotides were the predominant abnormalities in females with repeated pregnancy loss and unexplained infertility, even though autoantibodies to phospholipids and IgG as well as IgA isotypes were also detectable at somewhat lower incidence (9).

Our observations strengthen the argument that LA and specific autoantibodies correlate only poorly. This point was also made in recent reports by Cowchock et al. (4) and Rauch et al. (23). The latter failed to detect any correlation between LA antibody activity and antiplatelet, anti-DNA, anticardiolipin, antiphosphatidylethanolamine, antiphosphatidylserine, antiphosphatidylinositol, and antiphosphatidycholine activity. In contrast, antiplatelet activity was strongly correlated with antiphospholipid antibodies. Triplett et al. (18) also reported poor correlation between LA and antibodies directed against the negatively charged phospholipids cardiolipin, phosphatidylserine, phosphatidic acid, and phosphatidylinositol. They, however, reported an incidence of 73% IgG and/or IgM antibodies to phospholipids in their LA-positive study group. Such a relatively high incidence of positive autoantibodies can be expected based on their study design since autoantibody levels were considered elevated if they exceeded 2 standard deviations of only 30 control patients. In evaluating the distribution of autoantibody reactivity in a normal population for 45 isotypes, we recently demonstrated that the 99% confidence limit may represent 1.8–12 standard deviations of the mean, depending on isotype (17). Since the distribution of all phospholipid autoantibodies in a normal population is nonparametric, a 2 standard deviation cutoff will result in a disproportionate and, clinically probably inappropriate, high number of positive patients.

Finally, the data presented here suggest that LA screening is a deficient method to detect abnormal B-cell function in women with suspected reproductive failure due to abnormal autoimmunity. The detection of polyclonal B-cell abnormalities in such patients must be based on a broadly based profile. Cowchock et al. (4) have come to similar conclusions in evaluating patients with repeated abortions primarily in reference to antiphospholipid antibodies.

References

1. Lubbe WF, Butler WS, Palmer SJ, Lippins GC. Lupus anticoagulant in pregnancy. Br J Obstet Gynaecol 1984; 91:357–363.
2. Lubbe WF, Butler WS, Palmer SJ. Fetal survival after prednisone suppression of maternal lupus anticoagulant. Lancet 1983; 1:1361–1363.
3. Branch DW, Scott JR, Kochenour NK, Hershgold E. Obstetric complications associated with the lupus anticoagulant. N Engl J Med 1986; 313:1322–1326.
4. Cowchock S, Smith B, Gocial B. Antibodies to phospholipids and nuclear antigens in patients with repeated abortions. Am J Obstet Gynecol 1986; 155:1002–1010.
5. Harris EN, Chan JKH, Asherson RA, Aber VR, Gharavi AE, Hughes GRV. Thrombosis, recurrent fetal loss and thrombocytopenia. Predictive value of anti-cardiolipin antibody test. Arch Intern Med 1986; 146:2153–2156.
6. Branch W, Rote NS, Dostal D, Scott JR: Association of lupus anticoagulant with antibody against phosphatidylserine. Clin Immunol Immunopathol 1986; 39:298–302.
7. Cowchock FS, Somers D, Ruch D. ELISA for antibodies to phosphatidic acid (APA) compared to cardiolipin (ACA) in patients with repeated abortion (Abstract). Am J Reprod Immunol Microbiol 1987; 14:1.
8. Gleicher N, El Roeiy A, Confino E, Friberg J. Is endometriosis an autoimmune disease? Obstet Gynecol 1987; 70:115–122.
9. Gleicher N, El Roeiy A, Confino E, Friberg J: Reproductive failure because of autoantibodies: unexplained infertility and pregnancy wastage. Am J Obstet Gynecol 1989; 160:1376–1385.
10. El-Roeiy A, Gleicher N, Confino E, Friberg J, Dudkiewicz AB. Correlation between peripheral blood and follicular fluid: autoantibodies and impact on in vitro fertilization. Obstet Gynecol 1987; 70:163–170.
11. El-Roeiy A, Valesini G, Friberg J, et al. Autoantibodies and common idiotypes in men and women with sperm antibodies. Am J Obstet Gynecol 1988; 158:596–603.
12. Mannucci PM, Canciani MT, Ari DM. The varied sensitivity of partial thromboplastin and prothrombin time reagents in the demonstration of the lupus-like anticoagulant. Scand J Haematol 1979; 22:423–427.
13. Proctor RR, Rappaport SI. The partial thromboplastin time with kaolin: a simple screening test for first stage plasma clotting factor deficiencies. Am J Clin Pathol 1961; 36:212–216.
14. Bell WN, Alton HG. Brain extract as a substitute for platelet suspension in the thromboplastic test. Nature (Lond) 1954; 174:880–883.

15. Anonymous editorial. Lupus anticoagulant. Lancet 1984; 1:1157–1158.
16. Gleicher N, El-Roeiy A. Autoantibody levels of healthy females exceed those of healthy males. Submitted for publication.
17. El-Roeiy A, Gleicher N. Definition of abnormal autoantibody levels in an apparently healthy population. Obstet Gynecol 1988; 72:596–602.
18. Triplett DA, Brandt JT, Musgrave KA, Orr MT. The relationship between lupus anticoagulants and antibodies to phospholipid. JAMA 1988; 259:550–554.
19. Alving BM, Baker CS. Association of IgG cardiolipin antibody with thrombosis in patients with lupus anticoagulant (Abstract). Blood 1987; (Suppl 1):366A.
20. Lockshin MD, Druzin ML, Goei S. Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. N Engl J Med 1985; 313:152–156.
21. Shoenfeld Y, Rauch J, Massicote H, et al. Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. N Engl J Med 1983; 308:414–420.
22. Shoenfeld Y, Isenberg DA, Rauch J, Madio MP, Stollon BD, Schwartz RS. Idiotypic cross reactions of monoclonal human lupus autoantibodies. J Exp Med 1983; 158:718–730.
23. Rauch J, Meng Q-H, Tannenbaum H. Lupus anticoagulant and antiplatelet properties of human hybridoma autoantibodies. J Immunol 1987; 139:2598–2604.

Submitted for publication March 1990.
Revision submitted December 1990.

Intravenous Fat Emulsions and the Pancreas:

A Review

Andrew B. Leibowitz, M.D., Peggy O'Sullivan, M.S., R.D., and Thomas J. Iberti, M.D.

Parenteral nutrition is commonly employed to treat patients with pancreatic disease (1–5). The effect of this treatment on overall morbidity and mortality is controversial, but there is convincing evidence that parenteral nutrition improves the malnutrition, increased protein catabolism, and decreased protein synthesis that occur in these patients (2, 6–10).

Intravenous fat emulsions, once used solely as a means of preventing essential fatty acid deficiency, are now commonly prescribed as a major caloric source and often account for more than 50% of the intravenous calories supplied in many parenteral nutrition regimens. Although pancreatic disease is usually complicated by some degree of secretory dysfunction leading to glucose intolerance, the use of intravenous fat emulsions is often avoided, primarily for three reasons: intravenous fat emulsions may cause hypertriglyceridemia (11); type I and V hyperlipidemia have been demonstrated to cause pancreatitis (12); and pancreatitis may often be complicated by hypertriglyceridemia (13–15).

The object of this paper is to specifically review the use of intravenous fat emulsions in pancreatic disease.

Intravenous Fat Emulsions and Pancreatic Secretory Function

Studies of the effect of intravenous fat emulsions on pancreatic exocrine function in animals entail the creation of a controlled pancreatic fistula (16–21). Four of the five human studies were done on patients with pancreatic fistulas (21–24); the fifth was performed on normal human volunteers who had their duodenums intubated (25).

A total of five studies (16–20) were performed on a total of 27 mongrel dogs (see Appendix for details). To summarize, in the four studies (16, 17, 19, 20) that used Intralipid with or without background stimulation, no effect on fistula output or its constituent concentrations was seen. In the one study (18) that used Lipofundin, the bicarbonate and protein concentration of the output was increased but the total volume was not measured.

Matsuno et al. (21) studied alterations in exocrine and endocrine function of the pancreas following infusion of 10% Intralipid into five patients after pancreaticoduodenectomy and in five dogs with a chronic pancreatic fistula. In humans, pancreatic juice volume increased by 14.9% and bicarbonate concentration increased by 27.5% after 60 min of infusion, but amylase concentration did not change significantly. The dogs had no significant increase in volume output, bicarbonate concentration, or serum amylase at the end of the Intralipid infusion, but amylase output did increase.

Bivins et al. (22) reported a case of a 56-year-old woman who had complex vagotomy, Billroth I repair, and subsequent reconstruction that eventually left her with a pure pancreatic fistula from the duct of Wirsung. She was maintained on parenteral nutrition for eight days, including on day 6 400 cc and on day 8 450 cc of 10% safflower oil emulsion. Fistula output as well as bicarbonate and protein content did not increase with the administration of the intravenous fat emulsions. However, oral administration of fat after comple-

---

From the Department of Anesthesiology (ABL) and the Department of Surgery (TJI, PoS), The Mount Sinai Medical Center, New York, NY. Address reprint requests to Andrew B. Leibowitz, M.D., The Mount Sinai Medical Center, One Gustave L. Levy Place, Surgical Intensive Care Unit, Box 1264, New York, NY 10029.

tion of the parenteral nutrition course did increase fistula output as well as bicarbonate and protein concentration.

Klein et al. (23) studied the effect of infusing different components of parenteral nutrition on fistula output and bicarbonate and amylase concentration in a 29-year-old woman with a postoperative pancreatic fistula resulting from surgical excision of a benign islet cell tumor. Intralipid infusion was noted to increase fistula output from 800 cc to 940 cc per 24 hr, increase the bicarbonate concentration from 44.5 mEq to 54.5 mEq/L, and increase the amylase from 25,000 units to 32,000 units per 100 mL of fluid. The authors concluded that fat solutions should only be given in minimal amounts to provide essential fatty acids in patients with pancreatic fistulas. Unfortunately, this paper did not report the concentration, total amount, or rate of administration of the intravenous fat emulsions. Unlike the study of Bivins et al., the Intralipid was infused alone; this is important because the infusion of glucose and amino acids alone may decrease the pancreatic output and the concentration of its components, as well as possibly ameliorate the effect of intravenous fat emulsions.

Grundfest et al. (24) reported three patients with postoperative pancreatic fistulas. The patients were given alternating periods of isocaloric, isovolumetric, and isonitrogenous parenteral nutrition with and without fat. The effect of intravenous fat emulsions was studied by giving 1000 mL Intralipid 10% to two of the patients and 500 mL Intralipid 10% to the third. Fistula volume, amylase, lipase, and chloride concentrations, and trypsin activity were studied. None of these showed any significant increase with Intralipid administration. One of the patients was continued on an Intralipid-containing parenteral nutrition regimen and the fistula closed spontaneously; the other two patients required surgical closure.

Edelman and Valenzuela (25) studied the effects of graded doses of Intralipid on six healthy human volunteers. Pancreatic secretion was measured in a duodenal aspirate collected via a double-lumen tube and analyzed for amylase, trypsin, and bicarbonate. Graded amounts of Intralipid, 10%, were given at a rate of 2.5, 5.0, and 10.0 g/h. There was no change in any of these concentrations to any rate of administration, and the authors concluded that intravenous fat emulsions are safe to use in conditions where pancreatic stimulation is undesirable.

Conflicting Evidence. In sum, there is conflicting evidence in the literature on the effect of intravenous fat emulsions on pancreatic secretion. The studies reviewed lack uniformity in experimental design. Different types of intravenous fat emulsions have been given in the face of different background stimulus, possibly explaining the difference in outcome. Ten of the sixteen patients reported had no increase in fistula output or its constituent concentrations. The single case report of Klein omitted several important details, but nonetheless he concluded that intravenous fat emulsions should be administered only in doses large enough to prevent essential fatty acid deficiency. However, the patient's increase in output (from 33 cc/hr to 39 cc/hr) is approximately that reported by Matsuno and is not clinically significant.

Do Intravenous Fat Emulsions Cause Pancreatitis?

Three papers report a total of four patients thought to have developed acute pancreatitis as a direct result of intravenous fat emulsion infusion (26–28).

Case 1. Buckspan et al. (26) reported a case of an alcoholic man with a history of recurrent pancreatitis who was placed on parenteral nutrition while recovering from an acute bout of pancreatitis. After receiving 200 mL of intravenous fat emulsion, the patient developed pain, nausea, vomiting, and hyperamylasemia. The type of intravenous fat emulsion, its concentration, and rate of administration were not reported. Triglyceride levels were apparently not measured. After discontinuation of the intravenous fat emulsion, the patient recovered and no challenge with intravenous fat emulsions was attempted.

Case 2. Lashner et al. (27) reported the case of a 17-year-old man with Crohn's disease who was maintained on home parenteral nutrition including Intralipid 20%, 500 cc daily. The patient was admitted to the hospital with an acute abdomen, and exploratory laparotomy revealed pancreatitis. His postoperative course was uneventful and he continued to receive parenteral nutrition, including Intralipid 10%, 500 cc daily, without complication. Ten days into his recovery, with the patient's consent, Intralipid 20% was administered and acute pancreatitis recurred, presumably confirming the diagnosis of Intralipid 20%–induced pancreatitis. It should be noted that triglyceride metabolism may remain abnormal for several weeks after a bout of pancreatitis (31), and therefore this recurrence with Intralipid 20% does not necessarily prove the cause of the initial presentation. In addition, it seems unlikely that a 10% solution would be well tolerated and that simply an increase in the amount, not the type, of fat emulsion administered would so quickly lead to a recurrence of pancreatitis.

Cases 3 and 4. Noseworthy et al. (28) reported two cases of children, aged 10 and 13, with Crohn's disease, who had major intestinal resection and developed pancreatitis in the early postoperative period while receiving parenteral nutrition, including Intralipid. Both patients, however, were on high-dose prednisone, which has been implicated as a cause of pancreatitis (29). In addition, the dose of Intralipid given the second patient, 4 g/kg per day, was extremely high. Triglyceride levels were not reported and no challenge after recovery was attempted.

Intravenous fat emulsions are routinely used in virtually all patients presently receiving parenteral nutrition and yet only these four cases of intravenous fat emulsions causing pancreatitis have been reported. Note that three of these four patients had Crohn's disease. The other patient reported is the most poorly documented of the four cases.

Are Intravenous Fat Emulsions Safe For Patients with Pancreatitis?

Five papers address the use of intravenous fat emulsions to treat acute pancreatitis (8–10, 30, 31). One was an animal study and the other four were human studies.

Raasch et al. (30) studied intravenous fat emulsion infusion in 52 Sprague-Dawley rats that had experimentally induced pancreatitis. Thiry-seven survived 24 hr and were divided into two groups, receiving either parenteral nutrition without intravenous fat emulsion or parenteral nutrition with intravenous fat emulsions delivering 20% of the nonprotein calories. Twenty-eight rats were successfully entered into this stage of the protocol, 14 in each group. Rats living six days were killed. There was no significant difference in survival, urinary amylase excretion, pancreatic histology, or gross appearance of the animals between the two groups. The authors point out that the rat pancreatitis model poorly represents the pathophysiology of most human pancreatitis; therefore, these results are difficult to apply to clinical practice.

Gossum et al. (10) retrospectively reviewed 18 patients with severe acute pancreatitis admitted to the Intensive Care Unit of Toronto General Hospital; the cause was biliary in ten, alcohol in two, postoperative in two, unknown in four. All 18 received parenteral nutrition, including daily intravenous fat emulsion consisting of, on average, 100 g of fat from Nutralipid 10% or 20%. No ill effects of intravenous fat emulsion administration were noted. In fact, it was reported that the elevation in triglyceride levels was most closely associated with hyperglycemia and insulin resistance, not the amount of intravenous fat emulsion infused. Four patients were measured with a metabolic cart, and the respiratory quotient indicated that fat oxidation was as good as glucose oxidation. These observations led the authors to conclude that elevated triglyceride levels were not due to a specific abnormality of triglyceride metabolism, but were secondary to a global abnormality of energy metabolism.

Silberman et al. (8) studied 11 patients with acute pancreatitis, four prospectively and seven retrospectively. In six patients pancreatitis was attributed to alcohol consumption, in two to biliary tract disease, and in two to blunt abdominal trauma; one patient developed pancreatitis after cholecystectomy. Patients received parenteral nutrition including at least 150 g and as much as 350 g/day of Intralipid 10%. Patients received an average of 11 days of parenteral nutrition. Silberman et al. found no significant hypertriglyceridemia and noted no clinical or laboratory evidence to suggest any adverse effect of the intravenous fat emulsion.

The major problem with applying this study to clinical practice is the relatively short period of time patients were on parenteral nutrition, which may have led to an underestimate of side effect. However, this closely approximates the usual requirement and therefore, probably, the lack of significant adverse effect. In addition, it should be noted that the patients in this study were not as ill as those reported in the Gossum and Sitzman studies.

Sitzman et al. (9) prospectively studied 73 patients with severe pancreatitis treated with parenteral nutrition for two years. Pancreatitis was caused by alcohol in 41 patients, biliary tract disease in 15, hyperlipemia in 3, trauma in 5, after biliary tract manipulation in 3, and was idiopathic in 6. Patients were divided into three groups depending on their ability to tolerate fat or glucose as an energy source. The largest group, 44 patients, received glucose-based parenteral nutrition with twice-weekly infusion of Intralipid 10%, 500 mL. The second group comprised 20 patients who exhibited glucose intolerance and received 12%–50% of their calories by intravenous fat emulsion (type not reported). The third group, of 9 patients, received no intravenous fat emulsion at any time because of preexisting hypertriglyceridemia or thrombocytopenia. Overall, 87% of the patients received some regular infusion of intravenous fat emulsion. Parenteral nutrition was administered for an average of 29 days. No patient developed hypertriglyceridemia and none exhibited clinically apparent aggravation of the pancreatitis.

Notably, confirming the findings of the Gossum study, there was no correlation between the triglyceride levels and the amount of fat administered. The authors concluded that intravenous fat emulsions are safe to administer, malnutrition is common in these patients, failure to correct the malnutrition with parenteral nutrition correlates with decreased chance of survival, glucose intolerance is a common obstacle to this correction, and intravenous fat emulsions are therefore reasonable to use.

Durrington et al. (31) studied 33 patients at least six weeks following an attack of pancreatitis to determine their capacity to catabolize circulating triglycerides by administration of Intralipid 10%. The authors found that 4 of the 14 patients with persistent hypertriglyceridemia had impaired triglyceride clearance. They suggested that an intravenous lipid tolerance test may be useful in determining who might be vulnerable to a further attack of pancreatitis due to massive hypertriglyceridemia in certain circumstances. Interestingly, none of the patients studied, not even the patients with abnormal clearance, developed evidence of pancreatitis while undergoing this test. Serum glucose levels were not reported, which, in view of the findings of Gossum and Sitzman noted above, would have been of interest.

A significant body of evidence supports the routine use of intravenous fat emulsion in patients with pancreatitis who do not have an elevated triglyceride level. No reports have been published of adverse outcome secondary to use of intravenous fat emulsions in these circumstances.

Summary

There is conflicting evidence of the effect of intravenous fat emulsions on pancreatic secretion. Intralipid is a safe component of intravenous nutritional support in patients with pancreatic fistulas, though it may minimally increase the volume, as well as the bicarbonate and amylase concentrations, of the output. Intravenous fat emulsions may rarely cause pancreatitis; this may be more likely in patients with Crohn's disease, given that three of the four reported cases occurred in patients with Crohn's disease. It is unclear whether hypertriglyceridemia secondary to the intravenous fat emulsion is a prerequisite for this complication to occur. Intravenous fat emulsions appear to be a safe component of intravenous nutritional support for the patient with pancreatitis, based on multiple studies proving their safety in a total of nearly 100 patients. It seems prudent to avoid hypertriglyceridemia secondary to intravenous fat emulsions, as this alone is a cause of pancreatitis, albeit uncommon, in patients with abnormalities of triglyceride metabolism. However, hypertriglyceridemia resulting from parenteral nutrition may be caused by glucose intolerance and not intravenous fat emulsion administration.

Appendix

Burns and Stein (16) studied ten mongrel dogs with a surgically created pancreatic fistula. Pancreatic juice was collected during secretin ($n = 10$) or secretin plus cholecystokinin ($n = 4$) stimulation with and without Intralipid 10%, 50 cc administered over one hour. There was no significant change in fistula output or in concentration of bicarbonate, protein, amylase, lipase, or trypsin with administration of Intralipid.

Fried et al. (17) studied five mongrel dogs with a surgically created pancreatic fistula. Intralipid 10% was administered at a rate of 3.5 mL/kg per hour for two hours. Pancreatic output and protein concentration did not change. Furthermore, cholecystokinin, pancreatic polypeptide, and serum gastrin concentrations were similarly without significant change.

Konturek et al. (18) studied four mongrel dogs to compare the pancreatic secretory effects of natural amino acids, given as a mixture simulating serum albumin, or individually, and Lipofundin 20% (soybean emulsion 20%, soybean lecithin 1.5%, and xylitol 5%) 8g/hr administered intravenously or intraduodenally, with or without secretin. In addition, intravenous soybean lecithin 1.5% or xylitol in graded doses was administered. The solo administration of either of these Lipofundin components did not affect pancreatic secretion. However, Lipofundin 20% was found to significantly increase the bicarbonate and protein concentration of pancreatic output. Output volume itself was not measured. Lipofundin was not the intravenous fat emulsion administered in any other study.

Stabile and Debas (19) studied three mongrel dogs with surgically created pancreatic and gastric fistula. Each dog received graded doses of intravenous secretin plus cerulein (a cholecystokinin analog), and studies of pancreatic response to intravenous aminosyn, Intralipid (percent not reported), and glucose were performed. Individually administered Intralipid was found not to significantly affect pancreatic output or protein and bicarbonate concentration.

Stabile et al. (20) reported a similar study in five mongrel dogs. This study differed from the Stabile and Debas in two ways: cholecystokinin instead of cerulein was used, and Intralipid was administered in graded amounts at the specified percentage of 20%. Again, no effect on pancreatic output or protein and bicarbonate concentration was demonstrated.

References

1. Grant JP, James S, Grabowski V, Trexle KM. Total parenteral nutrition in pancreatic disease. Ann Surg 1984; 200:627–631.
2. Kirby DF, Craig RM. The value of intensive nutritional support in pancreatitis. J Parenter Enter Nutr 1985; 9:353–357.
3. Feller JH, Brown RA. Changing methods in the treatment of severe pancreatitis. Am J Surg 1974; 127:196–201.
4. Rowlands BJ. Intravenous nutrition for acute and chronic pancreatitis. In: Rombeau JC, Caldwell MD, eds. Parenteral nutrition. Philadelphia: WB Saunders 1986: 401–408.
5. Koruda MJ, Feuer ID. Pancreatic fistulas in nutritional medicine. In: Blackburn GL, Bell SJ, Mullen JL, eds. Nutritional medicine—a case management approach. Philadelphia: WB Saunders, 1989:39–43.
6. Goodman JT, Fisher JE. Parenteral nutrition in the treatment of acute pancreatitis—effect on complications and mortality. Ann Surg 1977; 186:651–658.
7. Sax HC, Warner BW, Talamini MA, et al. Early total parenteral nutrition in acute pancreatitis: lack of beneficial effects. Am J Surg 1987; 153:117–124.
8. Silberman H, Dixon NP, Eisenberg D. The safety and efficacy of a lipid based system of parenteral nutrition in acute pancreatitis. Am J Gastroenterol 1982; 77:494–497.
9. Sitzman JV, Steinborn PA, Zinner MJ, and Cameron JL. Total parenteral nutrition and alternate energy substrates in treatment of severe acute pancreatitis. Surg Gynecol Obstet 1989; 168:311–317.
10. Gossum AV, Lemoyne M, Greig PD, Jeejeebhoy KN. Lipid associated total parenteral nutrition in patients with severe acute pancreatitis. J Parenter Enter Nutr 1988; 12:250–255.
11. Adamkin DH, Gelke KN, Andrews BF. Fat emulsions and hypertriglyceridemia. J Parenter Enter Nutr 1984; 8:563–567.
12. Frederickson DS, Lees RS. Familial hyperlipoproteinemia. In: Stanby JB, Wyngarder JB, Frederickson DS, eds. The metabolic basis of inherited disease, 2nd ed. New York: McGraw-Hill, 1966:423–425.
13. Greenberger NJ, Hatch F, Druffey G, et al. Pancreatitis: a study of serum lipid alterations in 25 patients with acute pancreatitis. Medicine 1966; 45:161–174.
14. Cameron JL, Capuzzi DM, Zuidema GD, et al. Acute pancreatitis with hyperlipemia: evidence for a persistant defect in lipid metabolism. Am J Med 1974; 56:482–487.
15. Bush A, Bush J, Carlsen A, et al. Hyperlipidemia and pancreatitis. World J Surg 1980; 4:307–314.
16. Burns GP, Stein TA. Pancreatic enzyme secretion during intravenous fat infusion. J Parenter Enter Nutr 1987; 11:60–62.
17. Fried GM, Ogden WD, Rhea A, et al. Pancreatic protein secretion and gastrointestinal hormone release in response to parenteral amino acids and lipid in dogs. Surgery 1982; 92:902–905.
18. Konturek SJ, Tasler J, Cieszkowski M, et al. Intravenous amino acids and fat simulate pancreatic secretion. Am J Physiol 1979; 233:E678–684.
19. Stabile BE, Debas HT. Intravenous versus intraduodenal amino acids, fats, and glucose as stimulants of pancreatic secretion. Surg Forum 1981; 32:224–226.
20. Stabile BE, Borzatta M, Stubbs RS, Debas HT. Intravenous mixed amino acids and fats do not stimulate exocrine pancreatic secretion. J Physiol 1984; 246:G274–280.
21. Matsuno S, Miyashita E, Sasaki K, Sato T. Intraveous fat emulsion administration on exocrine and endocrine pancreatic function. Jpn J Surg 1981; 11:323–329.
22. Bivins BA, Bell RM, Rapp RP, et al. Pancreatic exocrine response to parenteral nutrition. J Parenter Enter Nutr 1984; 8:34–36.
23. Klein E, Shnebaum S, Ben-Ari G, Dreiling DA. Effects of total parenteral nutrition on exocrine pancreatic secretion. Am J Gastroenterol 1983; 78:31–33.
24. Grundfest S, Steiger E, Selinkoff P, Fletcher J. The effect of intravenous fat emulsions in patients with pancreatic fistula. J Parenter Enter Nutr 1980; 4:27–31.
25. Edelman K, Valenzuela JE. Effect of intravenous lipid on human pancreatic secretion. Gastroenterology 1983; 85:1063–1068.
26. Buckspan R, Woltering E, Waterhouse G. Pancreatitis induced by intravenous fat emulsion in an alcoholic patient. South Med J 1984; 77:252.
27. Lashner BA, Kirsner JB, Hanauer SB. Acute pancreatitis associated with high concentration lipid emulsion during total parenteral nutrition therapy for Crohn's disease. Gastroenterology 1986; 90:1039–1041.
28. Noseworthy J, Colodny AH, Eraklis A. Pancreatitis and intravenous fat: an association in patients with inflammatory bowel disease. J Pediatr Surg 1983; 18:269–272.
29. Nelp WB. Acute pancreatitis associated with steroid therapy. Arch Intern Med 1961; 108:702–710.
30. Raasch RH, Hak LJ, Benaim V, et al. Effect of intravenous fat emulsion in experimental acute pancreatitis. J Parenter Enter Nutr 1983; 7:254–256.
31. Durrington PN, Twentyman OP, Braganza JM, Miller JP. Hypertriglyceridemia and abnormalities of triglyceride catabolism persisting after pancreatitis. Int J Pancreat 1986; 1:195–203.

Submitted for publication January 1991.
Final revision received June 1991.

Synthetic Antiproteases in Acute Pancreatitis:

An Experimental Study

Marek Dobosz, M.D., Zbigniew Sledziński, M.D., Andrzej Babicki, M.D., Piotr Juszkiewicz, M.D., Andrzej Basiński, M.D., and Zdzisław Wajda, M.D.

Abstract

Acute pancreatitis was induced in 19 anesthetized dogs by retrograde injection of bile mixed with trypsin into the pancreatic duct. Two groups, of six animals each, were treated with intravenous infusion of synthetic antiproteases: gabexate mesilate and nafamostat mesilate in doses of 1 mg/kg per hr. One group of seven animals remained untreated. Two untreated dogs died during the experiment. All the treated dogs survived. Hemodynamic data were monitored hourly during a 6-hr observation period. In the untreated animals, cardiac output, mean arterial pressure, and left ventricular stroke volume decreased rapidly; an increase of pulmonary vascular resistance and systemic vascular resistance was observed. Synthetic antiproteases, given as a therapy, improved the hemodynamic parameters significantly and prevented the animals from developing shock. Gabexate mesilate and nafamostat mesilate seem to be of value in the treatment of experimentally produced acute pancreatitis in dogs.

ACUTE hemorrhagic pancreatitis is a disease with high morbidity and mortality. Hypovolemic shock and pulmonary insufficiency are largely responsible for early mortality (1, 2); later complications are usually due to pancreatic sepsis (3).

In the early stage of acute pancreatitis, activated pancreatic enzymes cause glandular tissue damage locally and cardiocirculatory, pulmonary, and renal dysfunctions (4–6) when absorbed into the circulation.

The use of antiproteases in the therapy of acute pancreatitis has been considered for many years, but their effectiveness is still controversial. Aprotinin, introduced as therapy in 1954, seems to be of no value in the treatment of acute pancreatitis (7), although Ruud et al. proposed that higher doses of aprotinin (8) may be useful.

Two new synthetic antiproteases, gabexate mesilate (FOY) and nafamostat mesilate (FUT-175), have been reported as of value in the treatment of acute experimental pancreatitis (9–12). Gabexate mesilate has also been used as a therapy in clinical conditions, but results were not uniform (13, 14).

The aim of this study was to investigate the hemodynamic effects of FUT-175 and FOY during the course of experimental acute pancreatitis.

Materials and Methods

Twenty-four male mongrel dogs weighing 14–22 kg were initially anesthetized with intramuscular ketamine hydrochloride 10 mg/kg (Ketalar, Parke & Davis). Supplementary doses of pentobarbital sodium were administered intravenously as required. Each dog was intubated and allowed to breath spontaneously. A catheter for monitoring arterial blood pressure was introduced into the femoral artery. Another catheter was inserted into the femoral vein for blood sampling and giving medicaments. A flow-directed

---

Presented in part at the First International Gastro-Surgical Club Joint Meeting, Aug. 31–Sept. 2, 1989, in Amsterdam.

From the Second Department of General Surgery and Department of Anaesthesiology, Medical School of Gdańsk, Gdańsk, Poland. Address reprint requests to Marek Dobosz, M.D., Second Department of General Surgery, Medical School of Gdańsk, Kieturakisa 1 St., 80-742 Gdańsk, Poland.

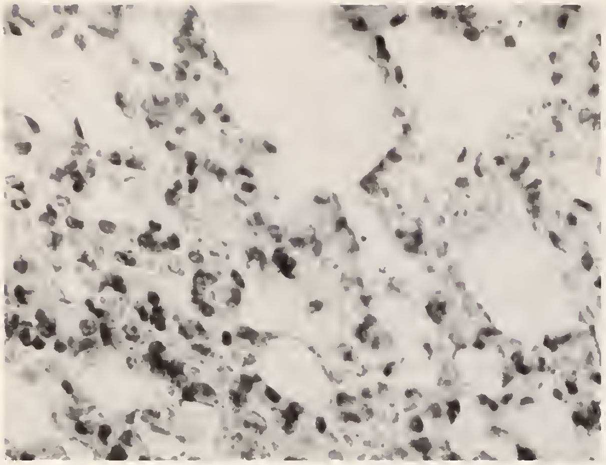


Fig. 1. Interstitial edema and thickened alveolar septa in lungs of untreated animals (group B).

Swan-Ganz catheter (Model 93-132-5F, American Edwards Laboratories) was floated into the pulmonary artery through the other femoral vein. The position of the Swan-Ganz catheter was confirmed by measuring the pressures during passage through the right side of the heart: vena cava, right atrium, right ventricle, and pulmonary artery. Arterial, central venous, pulmonary arterial, and pulmonary wedge pressures were recorded hourly throughout the experiment, using a pressure transducer (Model P23 Db Gould) and a Sirecust recorder (Model BS1). Cardiac output determinations were obtained by thermodilution, using a cardiac output computer (Tomel, Model COC-2).

The animals were divided into four groups.

Group A: Five dogs underwent laparotomy and duodenotomy only.

Group B: Seven dogs underwent laparotomy and duodenotomy, thereafter the pancreatic duct was cannulated and acute pancreatitis was induced by injection of 10 mL of bile mixed with 25 mg of trypsin (Serva) which had been incubated for 24 hr at 37°C. This mixture was injected into the cannulated pancreatic duct over 5 min at a pressure of 70 cm H_2O. All animals in groups A and B received 50 mL of 5% glucose intravenously during the experiment.

Group C: In six dogs, acute pancreatitis was induced as for Group B animals. Nafamostat mesilate (Torii and Co. Ltd.) was infused intravenously from 30 min after induction of pancreatitis until the end of the experiment at a dose of 1 mg/kg per hr in 50 mL of 5% glucose by constant infusion pump.

Group D: In six dogs, acute pancreatitis was induced as for Group B. Gabexate mesilate (Sanol Schwarz Gmbh) was then administered using the same route, dose, and timing as for Group C.

The solutions of FUT-175 and FOY were chemically and bacteriologically pure.

Unless an animal died sooner, an overdose of pentobarbitol was administered after 6 hours of observation. Pieces of lungs and pancreas were taken for histologic evaluation.

During the period of observation the following measurements were made hourly: cardiac output (CO), mean arterial pressure (MAP), heart rate (HR), central venous pressure (CVP), pulmonary arterial pressure (PAP), and pulmonary wedge pressure (PWP). Systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), and left ventricular stroke volume (LVSV) were calculated from these data.

Student's *t*-test was used to evaluate differences between the groups, p values < 0.05 being considered significant.

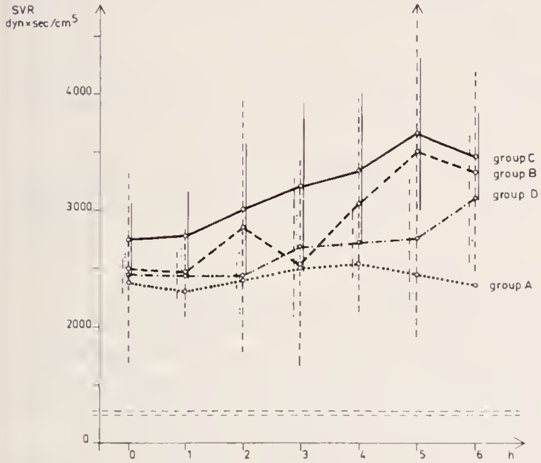
Results

Acute hemorrhagic pancreatitis, confirmed by histologic examination, developed in all animals in groups B, C, and D. Morphologic changes included necrosis of the pancreas, massively edematous retroperitoneum, and accumulation of hemorrhagic fluid within the peritoneal cavity. Histologic evaluation of lungs in the untreated animals (group B) revealed interstitial edema and thickened alveolar septa (Fig. 1). These changes were not seen in animals treated with nafamostat (group C) and gabexate mesilate (group D).

All the animals in Group B exhibited a rapid decrease in cardiac output, mean arterial pressure, and left ventricular stroke volume. The extent of these changes in the other groups of animals was less. Two animals in group B died while being observed. Systemic vascular resistance tended to increase in all animals with acute pancreatitis (Fig. 2) but this increase was not statistically significant. Pulmonary vascular resistance increased only in group B (untreated) dogs (Fig. 3).

Discussion

This study confirms the previously reported hemodynamic consequences of experimental acute pancreatitis (8, 15–17). These observations differ from those reported in patients with pancreatitis (4, 18), whose initial symptom is hyperdynamic shock. This difference may depend in part on when hemodynamic measurements are



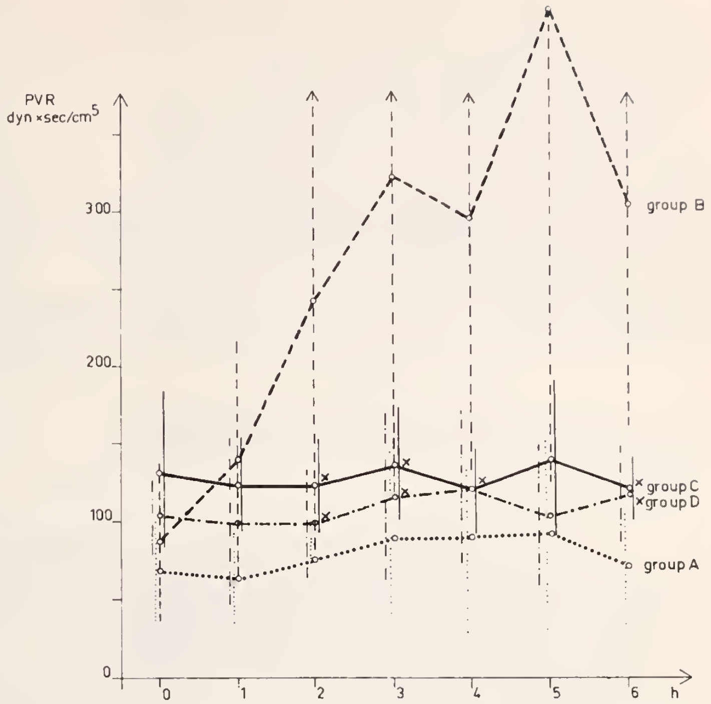


Fig. 2. (Left) Systemic vascular resistance (SVR) in dogs in group A (n = 5), control; group B (n = 7), untreated acute pancreatitis; group C (n = 6), acute pancreatitis treated with mafamostat mesilate (FUT-175); group D (n = 6), acute pancreatitis treated with gabexate mesilate (FOY). Mean values ± SD (vertical broken lines). No statistical significance among groups B, C, and D. Fig. 3. **Right** Pulmonary vascular resistance (PVR) in groups A, B, C and D. See legend for Fig. 2 for definition of groups. Mean values ± SD (vertical broken lines). $P < 0.05$ (x) compared to values of group B.

made after onset of pancreatitis—measurements are made much later in patients—and the amount of parenteral fluids administered prior to the clinical study.

During acute pancreatitis, active enzymes and toxins are known to be liberated from the inflamed pancreas (4, 19), These substances not only damage the pancreatic gland and the surrounding tissue, but activate proteolytic enzymes in plasma which have important roles in coagulation, fibrinolysis, and the kinin-kallikrein system (8, 20).

There is general agreement that adequate fluid administration is important in the early phase of acute pancreatitis (1, 19). Nonetheless, many patients with acute pancreatitis die of shock, despite apparently adequate fluid therapy (15).

Thus the logical aim of treatment in acute pancreatitis has been either to suppress and neutralize the release of pancreatic enzymes, or to inhibit the subsequent activation of the proteolytic enzyme systems in plasma. Although α_2-macroglobulin, a normal constituent of plasma, is a protease inhibitor, it does not address all the effects of the activated proteolytic enzymes, which can neutralize almost all the proteolytic enzymes. Unfortunately, in the severest forms of acute pancreatitis, the degree of saturation of α_2-macroglobulin is very high (21).

The beneficial effects of both the synthetic antiproteases used in our experiment may be due to their ability to inhibit kallikrein, trypsin, trypsin α_2-macroglobulin complex, thrombin, plasmin, C_1-esterase, and phospholipase A_2 (11, 21).

The inhibition of phospholipase A_2 may be an important therapeutic goal. It is this enzyme, in its active form, which is responsible for autodigestion of pancreatic gland and peripancreatic fatty tissue (22). Moreover, phospholipase A_2 has been implicated as an agent that causes direct damage to the pulmonary capillary endothelium and leads to lung injury (5). Our untreated animals all demonstrated increased pulmonary vascular resistance, together with interstitial edema and thickened alveolar septa in the lungs. These findings may be the equivalent of the development in humans of adult respiratory distress syndrome in the early stage of acute pancreatitis (23). The synthetic antiproteases used in our experiment reduced this dangerous complication.

Major shortcomings of this study are the use of the dog as the experimental animal and the short interval between experimental induction of acute pancreatitis and initial administration of the antiproteolytic agents, which precludes meaningful comparison to human pancreatitis. In the clinic the interval is usually longer.

However, the clear evidence of protection offered by these agents during the early phases of acute pancreatitis induced experimentally in dogs suggests that early administration of antiproteolytic therapy may be useful in some patients. A randomized clinical trial will be necessary to evaluate the early use of these new synthetic proteases in acute pancreatitis.

Acknowledgments

Gabexate mesilate was kindly provided by Sanol Schwarz Gmbh. We also thank Torii and Co. Ltd. for the generous gift of nafamostat mesilate.

References

1. Ranson JHC. Acute pancreatitis: pathogenesis, outcome and treatment. Clin Gastroenterol 1984; 13:843–863.
2. Ranson JHC, Turner JW, Roses DF, Rifkind KM, Spencer FC. Respiratory complications in acute pancreatitis. Ann Surg 1974; 179:557–566.
3. Frey CF, Bradley EL, Beger HG. Progress in acute pancreatitis. Surg Gynecol Obstet 1988; 167:282–286.
4. Beger HG, Bittner R, Büchler M, Hess W, Schmitz JE. Hemodynamic data pattern in patients with acute pancreatitis. Gastroenterology 1986; 90:74–79.
5. Guice KS, Oldham KT, Wolfe RR, Simon RH. Lung injury in acute pancreatitis: primary inhibition of pulmonary phospholipid synthesis. Am J Surg 1987; 153:54–61.
6. Levy S, Geller R, Hymovitch S. Renal failure in dogs with experimental acute pancreatitis: role of hypovolemia. Am J Physiol 1986; 251:969–977.
7. Imrie CW, Benjamin IS, Ferguson JC, et al. A single-centre double-blind trial of Trasylol therapy in primary acute pancreatitis. Br J Surg 1978; 65:337–341.
8. Ruud TE, Aasen AO, Stadaas JO, Aune S. Effects on peritoneal proteolysis and hemodynamics of prophylactic and therapeutic infusions of high doses of aprotinin in experimental acute pancreatitis. Scand J Gastroenterol 1986; 21:1011–1017.
9. Takasugi S, Yonezawa H, Ikei N, Kanno T. Prevention of acute experimental pancreatitis in rats and dogs by intraduodenal infusion of a synthetic trypsin inhibitor. Digestion 1982; 24:36–41.
10. Niederau C, Crass R, Silver G, Ferrel LD, Grendell JH. Therapeutic regimens in acute experimental pancreatitis. Effects of hydration, oxygenation, peritoneal lavage, and a potent protease inhibitor. Gastroenterology 1988; 95:1648–1657.
11. Iwaki M, Ino Y, Motoyoshi A, et al. Pharmacological studies of FUT-175, nafamostat mesilate. V. Effects on the pancreatic enzymes and experimental acute pancreatitis in rats. Jpn J Pharmacol 1986; 41:155–162.
12. Satake K, Koh I, Nishiwaki H, Umeyama K. Toxic products in hemorrhagic ascitic fluid generated during experimental acute hemorrhagic pancreatitis in dogs and a treatment which reduces their effect. Digestion 1985; 32:99–105.
13. Tanaka N, Tsuchiya R, Ishii K. Comparative clinical study of FOY and trasylol in acute pancreatitis. Adv Exp Med Biol 1979; 120B:367–378.
14. Freise J, Melzer P, Schmidt FW, Horbach L. Gabexat mesilat in der Behandlung der akuten Pankreatitis Ergebnisse der hannoverschen multizentrischen Doppelblindstudie mit 50 Patienten. Zentralbl Gastroenterol 1986; 24:200–211.
15. Horton JW, Burnweit CA. Hemodynamic function in acute pancreatitis. Surgery 1988; 103:538–546.
16. Hjelmqvist B, Ohlsson K. Aronsen KF. Protease-antiprotease imbalance, hemodynamic and regional blood flow changes in experimental pancreatitis. Scand J. Gastroenterol 1986; 21(Suppl 126):8–11.
17. Martin DT, Steinberg SM, Koplovic R, Carey LC, Cloutier CT. Crystalloid versus colloid resuscitation in experimental hemorrhagic pancreatitis. Surg Gynecol Obstet 1984; 159:445–449.
18. Bradley EL, Hall JR, Lutz J, Hammer L, Lattouf O. Hemodynamic consequences of severe pancreatitis. Ann Surg 1983; 198:130–133.
19. Thomas PD. Acute pancreatitis. Anaesth Intensive Care 1985; 13:249–257.
20. Ruud TE, Aasen AO, Kierulf P, Stadaas JO, Aune S. Studies on the plasma kallikrein-kinin system in peritoneal exudate and plasma during experimental acute pancreatitis in pigs. Scand J Gastroenterol 1985; 20:877–882.
21. Freise J, Schmidt FW, Magerstedt P, Schmid K. Gabexate mesilate and camostate: new inhibitors of phospholipase A_2 and their influence on the alpha-amylase activity in serum of patients with acute pancreatitis. Clin Biochem 1985; 18:224–229.
22. Keynes M. Heretical thoughts on the pathogenesis of acute pancreatitis. Gut 1988; 29:1413–1423.
23. Shoemaker WC, Appel PL. Pathophysiology of adult respiratory distress syndrome after sepsis and surgical operations. Crit Care Med 1985; 13:166–172.

Submitted for publication February 1990.
Final revision submitted July 1990.

Carcinoma of the Gallbladder:

Review of the Literature and Report on 56 Cases at the Beth Israel Medical Center

Leigh H. Nadler, M.D., and Charles K. McSherry, M.D.

Abstract

Fifty-six patients with carcinoma of the gallbladder were treated at the Beth Israel Medical Center, New York City, during the 17-year period 1969–1987. The disease was most common among elderly women with cholelithiasis. Of the 56, 42 patients (75%) had liver metastases at operation; therefore, the majority underwent only exploratory laparotomy and biopsy. In 50 patients (89%), the tissue diagnosis was adenocarcinoma. Cholecystectomy was performed in 19 patients. Long-term survival occurred in patients with an incidental diagnosis of carcinoma of the gallbladder. The average survival in 44 patients for whom data were available, including long-term survivors, was 20 months. Of this group, 5 patients (11.3%) were still alive 5 years after diagnosis. Excluding those with an incidental diagnosis of gallbladder cancer, 1 patient (2.2%) survived longer than 5 years after diagnosis. Survival data in 3 of 4 patients who underwent extensive surgical treatment, such as liver wedge resection or hepatic lobectomy, averaged 23 months. Although no defined protocols were used, the addition of adjuvant chemotherapy or radiation therapy in patients treated surgically appeared to improve survival. Five patients survived more than 5 years after diagnosis, and 2 patients were alive after 17 years.

Carcinoma of the gallbladder is one of the most rapidly fatal forms of carcinoma. Much has been written on this subject to hasten preoperative diagnosis, define adequate treatment, and improve survival. Carcinoma of the gallbladder is responsible for nearly 6,500 deaths per year in the United States and is the fifth most common gastrointestinal malignancy (1, 2). It was first described by Maximillian DeStoll in 1771 (3). Because of the lack of specific symptoms, late diagnosis and ineffective treatment are typical (4). The signs and symptoms associated with resectable carcinoma of the gallbladder are the same as those associated with nonmalignant disease of this organ. In an attempt to determine an association between method of diagnosis, treatment, and survival, we reviewed cases of carcinoma of the gallbladder from December 1969 to January 1987 at the Beth Israel Medical Center, New York City.

Methods and Materials

The records of 56 patients with the diagnosis of carcinoma of the gallbladder were reviewed (Table 1). Survival time from date of diagnosis was determined in 44 patients. Four patients are still alive. Attempts to determine survival of 12 patients by phone or mail were unsuccessful; these patients are not included in the survival data.

Results

A history of chronic gallbladder disease, with or without associated gallstones, was present in 24 of 30 patients with available data. Nine patients had symptoms for over 10 years, including 2 who had a 30-year history of symptoms. Data concerning the presence of gallstones was avail-

---

From the Department of Surgery, Beth Israel Medical Center, and the Mount Sinai School of Medicine (CUNY), New York, NY. Address reprint requests to Charles K. McSherry, M.D., Director, Department of Surgery, Beth Israel Medical Center, First Avenue at 16th Street, New York, NY 10003.

TABLE 1
Demographic Data

	n	Age range (Mean) (years)
Women	38	51–85 (67)
Men	18	36–97 (70)

able in 45 patients. Cholelithiasis was present in 42 of 45 patients for whom data were available. No stones were reported in 3 cases.

The common initial signs and symptoms are listed in Table 2. Unusual initial presentations included a draining sinus in the right hypochondrium, pelvic mass, upper gastrointestinal bleeding, gastric outlet obstruction, intestinal obstruction, perforated viscus, and diabetic ketoacidosis. On admission, 23% of patients were found to have obstructive jaundice; 18% had acute cholecystitis. Abnormal liver function tests were found in 75% (42/56) of patients, and carcinoembryonic antigen (CEA) level was elevated to greater than 12 ng/mL in 4 of 4 patients in whom the test was performed.

Diagnostic Procedures. Although the results of various radiographic studies were abnormal, only four procedures—abdominal ultrasound, abdominal computerized axial tomography (CTT), percutaneous transhepatic cholangiography (PTC), and angiography—suggested the diagnosis of carcinoma of the gallbladder. Although performed in only 4 patients, all celiac angiograms were compatible with a diagnosis of gallbladder cancer. Abdominal ultrasound and CTT are widely used today; however, in this study, only 13% (3/23) of sonograms and 28% (4/14) of CTTs were diagnostic of carcinoma of the gallbladder (Fig. 1). Although PTC was abnormal in all 12 patients tested, the findings were not suggestive of gallbladder cancer.

Operative Treatment. The majority of patients underwent exploratory laparotomy with tumor biopsy because of the extent of the lesion (Table 3); 3 patients did not undergo surgical intervention because of their advanced disease and general medical condition. Only 8 of 56 patients (14%) had no metastatic disease at the time of surgical exploration. Of the sites of metastases (Table 4), that to the liver was most often secondary to direct extension from the gallbladder. Unusual sites included the ovary, small intestine, transverse mesocolon, and costal margin.

TABLE 2
Common Clinical Features

Symptom	% of patients
Pain	62
Jaundice	53
Weight loss	53
Anorexia	48
Mass	32
Hepatomegaly	32
Ascites	32

Adjuvant Therapy. Twenty-two patients who underwent surgical intervention for either diagnostic biopsy or intended surgical cure received adjuvant therapy (radiation therapy [R] and/or chemotherapy [C]). Survival data was available for 20 of these patients (Table 5). Those treated with radiation plus combination chemotherapy (5-fluorouracil[5-FU] and mitomycin C) had similar presenting symptoms and laboratory findings when compared with those patients treated with chemotherapy alone. Excluding patients with an incidental diagnosis of carcinoma of the gallbladder in whom long-term survival is expected, this data suggests an apparent benefit with the addition of adjuvant chemotherapy or radiation therapy to surgical treatment alone. Although longer survival was seen in patients who received surgery plus radiation therapy, the total number of patients in this group is too small to suggest this mode of therapy is best. It is important to point out that data regarding the specific protocols used for adjuvant chemotherapy or radiation treatment were not available for review, and therefore it is difficult to compare the different adjuvant therapies.

Histopathology. A histologic diagnosis was recorded in 49 patients. Adenocarcinoma was present in 90% (44/49) of patients. The remainder had squamous cell or adenosquamous cell carcinoma. The extent of gallbladder involvement was recorded in 16 patients. Involvement was diffuse in 44% (7/16), limited to the fundus in 19% (3/16), and involved the ampulla and neck in 38% (6/16). An incidental diagnosis of carcinoma of the gallbladder followed microscopic examination in 5 patients in whom the gallbladder was removed for acute cholecystitis or jaundice. Two of these patients are still alive.

Length of Survival. Survival data was available in 44 patients. One case was diagnosed at autopsy. Of this group, 13 patients survived longer than one year. The overall five-year survival rate is 11.3% (5/44), which is unusual. Long-term survival was more apt to occur in patients with an incidental diagnosis of carcinoma of the gallbladder. Excluding 4 patients in whom gallbladder cancer was diagnosed incidentally following cholecystectomy, only 1 patient (2.2%) survived longer than 5 years.

Women had a proportionately better survival than men at 3–12 months after diagnosis (Fig. 2). Length of survival was similar at 15 months. Men tended to have an advantage in long-term survival (Fig. 3).

Since most pathology reports did not detail the level of penetration of tumor into the wall, and surgical reports did not describe the extension of tumor into the liver, staging according to the American Joint Committee on Cancer was not possible (5).

Discussion

Carcinoma of the gallbladder is found in 0.5% of routine autopsies and in 1.0% of patients with gallstones, most often in women over age 65 (6, 7). The higher frequency of gallbladder cancer in the female population is consistent with the increased incidence of calculous biliary disease in women. (3). The incidence of carcinoma of the gallbladder in patients operated on for biliary tract disease averages 1.9% (range, 0.5%–6.5%) (4, 8).

Although generally a disease of elderly women, gallbladder cancer has been reported in children. The youngest patient reported is an 11-year-old Navajo Indian girl without gallstones (9).

Ethnic and regional differences are well noted in the literature (10–14). The highest incidence of gallbladder cancer was found in the Hispanic and American Indian population of New Mexico (11, 12).

The association of cholelithiasis with carcinoma of the gallbladder has been well documented (15–21). In a cumulative series of 1,419 patients reported by Do Carmo et al., carcinoma of the gallbladder occurred in 0.4% of patients with known gallstones for at least 10 years (20).

Early cholecystectomy in patients with asymptomatic disease may be more appropriate to prevent the complications of benign disease than to prevent the development of carcinoma. Schoenfield (6) recommended elective cholecystectomy in younger patients. Acute cholecystitis, pancreatitis, or jaundice may be the initial symptom in 20% of patients with cholelithiasis. Delaying surgery was likely to increase operative mortality as age-related diseases of other organs increased. Glenn and McSherry noted that cardiovascular disease, especially myocardial infarction, was the most frequent cause of death in elderly patients undergoing biliary surgery (22). Recent reports indicate operative mortality rates of 1% or less for acute and chronic cholecystitis (23). In one series of 10,749 cholecystectomy patients, operative mortality rates for acute and chronic cholecystitis were 1.2% and 0.4%, respectively (24).

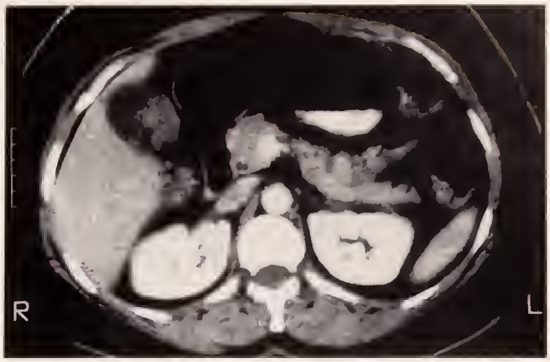


Fig. 1. CTT scan demonstrating carcinoma of the gallbladder.

The lack of specific signs or symptoms fails to distinguish malignant and benign disease or to identify malignancy at an early, resectable stage (25–27). Symptomatic patients usually have advanced disease because of metastases to hilar lymph nodes before the bile ducts are obstructed (28).

Patients with gallbladder cancer may present with acute cholecystitis and nonvisualization of the gallbladder on cholescintigraphy. This may be due to cystic duct obstruction from either stones, tumor, or decreased gallbladder compliance from tumor infiltration of the gallbladder wall (29). Piehler and Crichlow found acute cholecystitis in 16% of 534 patients with carcinoma of the gallbladder (4).

Laboratory and radiographic studies have not been consistently helpful in the early diagnosis of carcinoma of the gallbladder. Serum CEA is

TABLE 3
Operative Treatment

	Treatment (n)		
Procedure	Elsewhere	BIMC	Total
Cholecystectomy	2	17*	19
Cholecystostomy	—	5	5
Exploratory, laparotomy, and biopsy	—	29	29
No operation	—	3	3
Total	2	54	56

* Four patients also had liver wedge resection or lobectomy.

TABLE 4
Metastases

Site	% of patients*
Liver	75
Omentum	25
Porta hepatis	23
Common duct	12
Duodenum	12
Colon	11
Peritoneum	11
Abdominal wall	7
Pancreas	5
Stomach	5

* 14% of patients had no metastases.

a nonspecific indicator of gastrointestinal malignancy. It may be helpful in the diagnosis of carcinoma of the gallbladder (30); however, it was performed in only four patients in this study. Biliary ultrasound, CTT, PTC, endoscopic retrograde cholangiography, upper gastrointestinal series, and angiography have been reported by others to assist in establishing the diagnosis of carcinoma of the gallbladder (4, 31–35). Selective and highly selective angiography is reported to have the highest diagnostic accuracy of all radiographic procedures (4, 36).

In a study of 151 patients, Fahim et al. reported the modes of spread of gallbladder cancer as lymphatic, neural, vascular, intraductal, or intraperitoneal seeding, or by direct extension (37, 38). Lymph node metastases to the porta hepatis enlarged sufficiently to produce obstructive jaundice, even without obvious liver involvement (37); 75% of our patients had liver metastases.

The surgical treatment of carcinoma of the gallbladder may include cholecystectomy, liver wedge resection, lymph node dissection, lobectomy, or bypass in advanced disease (39–42). Only 5% of all operations for carcinoma of the gallbladder involve surgical procedures more extensive than simple cholecystectomy (4). An adequate operation for early gallbladder cancer should consist of cholecystectomy, wedge resection of the liver with a margin of tissue free of tumor, and adequate nodal dissection of the hepatoduodenal ligament (37). Palliative surgery is desirable in the late stages to relieve intestinal obstruction or obstructive jaundice.

TABLE 5
Survival by Mode of Therapy, 53 Patients with Carcinoma of the Gallbladder

Method of treatment	No. of pts	Survival	
		No.*	Period
Surgery alone	31	24	26.7 mo
Surgery/chemotherapy			
w/5-FU	10	9	11.5 mo
w/5-FU and mitomycin	1	1	7 wk
Surgery/RT	3	3	23 mo
Surgery/5-FU/RT	4	3	10.3 mo
Surgery/5-FU/RT and mitomycin	4	4	12.2 mo

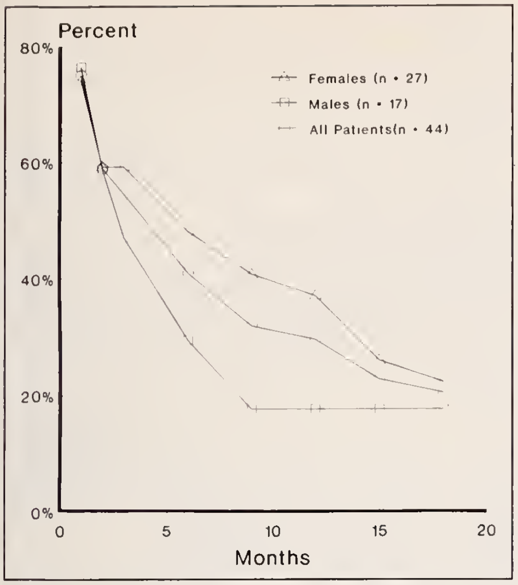
RT, radiotherapy.
* Number of patients for whom survival data available.

The improvement in survival following radical resection is controversial. Although Nevin concluded that the prognosis of patients with transmural and cystic node involvement may be improved with right hepatic lobectomy and lymphadenectomy (39), others have not found radical surgery to improve survival (19, 43).

Considering the large number of patients who are not candidates for radical resection, the combination of radiation and chemotherapy should be further evaluated. Although adjuvant chemotherapy or radiation therapy is considered to have minimal effect on survival (3, 44), others support the palliative benefits of adjuvant therapy (45–47). Evidence supporting a combination of treatments, including surgery, chemotherapy, and radiotherapy, may enhance survival and improve palliation (44, 48–51). Additional prospective clinical studies are necessary to further evaluate the use of adjuvant chemotherapy and radiation therapy.

Summary

The results of a retrospective review of 56 patients with carcinoma of the gallbladder confirm the extremely poor prognosis of this disease. Five cases were diagnosed incidentally on routine microscopic examination of the gallbladder following its removal for symptomatic calculous biliary disease. A small percentage of patients with localized disease may be recognized at the time of laparotomy and may undergo curative resection. Visual inspection of all gallbladder specimens, with frozen section of suspicious areas, is recommended to improve the prognosis in patients with early noninvasive gallbladder cancer. Excluding patients with an incidental diagnosis of carcinoma of the gallbladder, this review suggests the apparent benefit of the addition of adjuvant chemotherapy or radiation therapy to surgical treatment. Further studies are necessary to more clearly define possible benefits of adjuvant therapy.



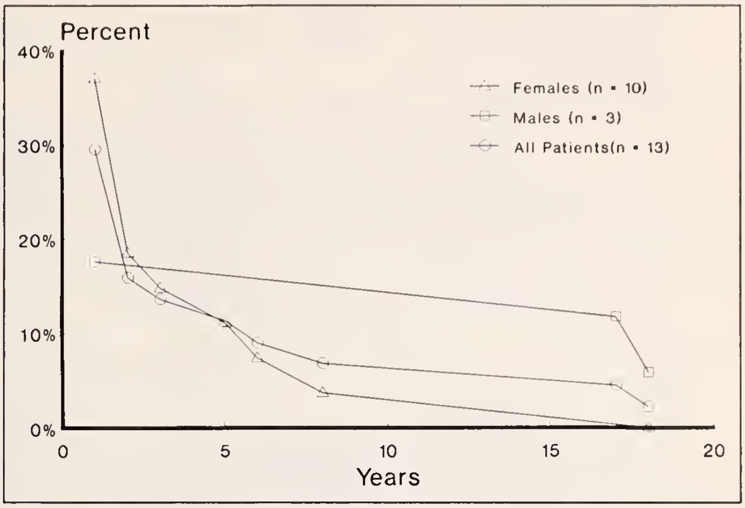


Fig. 2. Left Short-term survival by sex, 1–18 months, 44 patients for whom data available. **Fig. 3. Right** Long-term survival by sex, 1–18 years, 13 patients for whom data available. One patient died 18 years following incidental diagnosis of gallbladder cancer. Current survivors include one each at 6, 8, and 17 years following surgery.

Acknowledgments

Thanks to Johanna L. Drickman, M.D., and Shirley Nadler for reviewing the manuscript.

References

1. Holmes SL, Mark JB. Carcinoma of the gallbladder. Surg Gynecol Obstet 1971; 133:561–564.
2. Schwartz SI. Gallbladder and extrahepatic biliary system. In: Surgery. 4th ed. New York: McGraw-Hill, 1984: 1332–1333.
3. Ram MD. Carcinoma of the gallbladder. Surg Gynecol Obstet 1971; 132:1044–1048.
4. Piehler JM, Crichlow RW. Primary carcinoma of the gallbladder. Surg Gynecol Obstet 1978; 147:929–942.
5. American Joint Committee on Cancer. Manual for staging of cancer. 3rd ed. Philadelphia: JB Lippincott, 1988:93–95.
6. Schoenfield LJ. Gallstones and other biliary diseases. Clin Symp 1982; 34:2.
7. Kimura W, Nagai H, Kuroda A, Morioka Y. Clinicopathologic study of asymptomatic gallbladder carcinoma found at autopsy. Cancer 1989; 64:98.
8. Gupta S, Udupa KN. Primary carcinoma of the gallbladder; a review of 328 cases. J Surg Oncol 1980; 14:35–44.
9. Rudolph R, Cohen JJ. Cancer of the gallbladder in an 11 year old Navajo girl. J Pediatr Surg 1972; 7:66–67.
10. Boss LP, Lanier AP, Dohan PH, Bender TR. Cancers of the gallbladder and biliary tract in Alaskan natives: 1970–1979. J Natl Cancer Inst 1982; 69:1005–1007.
11. Devor EJ, Buechley RW. Gallbladder cancer in Hispanic New Mexicans. Cancer 1980; 45:1705–1712.
12. Morris DL, Buechley RW, Key CR, Morgan MV. Gallbladder cancer among American Indians in tricultural New Mexico. Cancer 1978; 42:2472–2477.
13. Reichenback DD. Autopsy incidence of diseases among southwestern American Indians. Arch Pathol 1967; 84:81–86.
14. Rios-Dalenz J, Takabayashi A, Henson DE, Stom BL, Soloway RD. Cancer of the gallbladder in Bolivia: suggestions concerning etiology. Am J Gastroenterol 1985; 80:371–375.
15. Hart J, Modan B, Shani M. Cholelithiasis in the aetiology of gallbladder neoplasms. Lancet 1971; 1:1151–1153.
16. Parkash O. On the relationship of cholelithiasis to carcinoma of the gallbladder and on the sex dependency of carcinoma of the bile ducts. Digestion 1975; 12:129–133.
17. Roberts JW, Daugherty SF. Primary carcinoma of the gallbladder. Surg Clin North Am 1986; 66:743–749.
18. Kuroda A, Imanari T. Indication of silent stones for surgery viewed from relationship between cholelithiasis and gallbladder cancer. Gastroenterol Jpn 1979; 14:636–637.
19. Glenn F, Hays DM. The scope of radical surgery in the treatment of malignant tumors of the extrahepatic biliary tract. Surg Gynecol Obstet 1954; 99:529–541.
20. Do Carmo M, Perpetuo MO, Valdivieso M, et al. Natural history study of gallbladder cancer: a review of 36 years experience at M.D. Anderson Hospital and Tumor Institute. Cancer 1978; 42:330–335.
21. Maringhini A, Moreau JA, Melton J, Hench VS, Zinsmeister AR, DiMagno EP. Gallstones, gallbladder cancer, and other gastrointestinal malignancies. Ann Intern Med 1987; 107:30.
22. Glenn F, McSherry CK. Calculous biliary tract disease. Curr Prob Surg 1975;June.
23. Wetter LA, Way LW. Surgical therapy for gallstone disease. Gastroenterol Clin North Am 1991; 20:157.
24. McSherry CK. Cholecystectomy: the gold standard. Am J Surg 1989; 158:174–178.
25. Marsidi PJ, Anwar M, Halsted GO. Primary carcinoma of the gallbladder. J Med Soc NJ 1979; 76:347–349.
26. Barr LH, Wright FH. Carcinoma of the gallbladder. Am Surg 1984; 50:275–276.
27. Koo J, Wong J, Cheng F, Ong G. Carcinoma of the gallbladder. Br J Surg 1981; 68:161–165.
28. Bismuth H, Malt RA. Carcinoma of the biliary tract. N Engl J Med 1979; 301:704–706.
29. Colletti PM, Ralls PW, Siegel ME, Halls JM. Cholescin-

tigraphy in gallbladder carcinoma. Clin Nucl Med 1986; 11:270–273.

30. Albores-Saavedra J, Nadji M, Morales AR, Henson DE. Carcinoembryonic antigen in normal, preneoplastic and neoplastic gallbladder epithelium. Cancer 1983; 52:1069–1072.
31. Yeh HC. Ultrasonography and computed tomography of carcinoma of the gallbladder. Radiology 1979; 133:167–173.
32. Koga A, Yamauchi S, Izumi Y, Hamanaka N. Ultrasonographic detection of early and curable carcinoma of the gallbladder. Br J Surg 1985; 72:728–730.
33. Collier A, Carr D, Hemingway A, Blumgart LH. Preoperative diagnosis and its effect in the treatment of carcinoma of the gallbladder. Surg Gynecol Obstet 1984; 159:465–470.
34. Thorsen M, Quiroz F, Lawson T, Smith D, Foley W, Stewart E. Primary biliary carcinoma: CT evaluation. Radiology 1984; 152:479–483.
35. Ogoshi K, Niwa M, Hara Y, Nebel OT. Endoscopic pancreatocholangiography in the evaluation of pancreatic and biliary disease. Gastroenterology 1973; 64:210–216.
36. Sato T, Watanabe K, Saitoh Y, Koyoma K, Suda Y. Selective arteriography for gallbladder diseases. Arch Surg 1969; 99:598–605.
37. Fahim RB, Ferris DO, McDonald JR. Carcinoma of the gallbladder. Arch Surg 1963; 86:176–182.
38. Fahim RB, McDonald JR, Richards JC, Ferris DO. Carcinoma of the gallbladder: a study of its modes of spread. Ann Surg 1962; 156:114–124.
39. Nevin JE, Moran TJ, Kay S, King R. Carcinoma of the gallbladder: staging, treatment, and prognosis. Cancer 1976; 37:141–148
40. Sheinfeld W. Cholecystectomy and partial hepatectomy for carcinoma of the gallbladder with local liver extension. Surgery 1947; 22:48.
41. Brasfield RD. Right hepatic lobectomy for carcinoma of the gallbladder: a five year cure. Ann Surg 1961; 153:563–566.
42. Nakamura S, Sakaguchi S, Suzuki S, Muro H. Aggressive surgery for carcinoma of the gallbladder. Surgery 1989; 106:467–473.
43. Evander A, Ihse I. Evaluation of intended radical surgery in carcinoma of the gallbladder. Br J Surg 1981; 63:158–160.
44. Jonsson P, Petterson BO. Carcinoma of the gallbladder—a natural history type of study. J Surg Oncol 1982; 21:215–218.
45. Shukla VK, Khandelwal C, Roy SK, Vaidya MP. Primary carcinoma of the gallbladder: a review of a 16-year period at the University Hospital. J Surg Oncol 1985; 28:32–35.
46. Smoron GL. Radiation therapy of carcinoma of the gallbladder and biliary tract. Cancer 1977; 40:1422–1424.
47. Houry S, Schlienger M, Huguier M, Lacaine F, Penne F, Laugier A. Gallbladder carcinoma: role of radiation therapy. Br J Surg 1989; 76:448–450.
48. Treadwell TA, Hardin WJ. Primary carcinoma of the gallbladder: the role of adjuvant therapy in its treatment. Am J Surg 1976; 132:703–706.
49. Whetstone MR, Saltzstein EC, Mercer LC. Demographic characteristics of gallbladder cancer in an area endemic for biliary calculi. Am J Surg 1986; 152:728.
50. Wanebo HJ, Castle WN, Fechner RE. Is carcinoma of the gallbladder a curable lesion? Ann Surg 1982; 195:624–631.
51. Todoroki T, Iwasaki Y, Okamura T, Nagoshi K, Asakura H, Nakano M, Inada T, Tsunemoto H, Umegaki Y, Nishimura A, Nakano M, Sato H. Intraoperative radiotherapy for advanced carcinoma of the biliary system. Cancer 1980; 46:2179–2184.

Submitted for publication June 1989.
Final revision submitted April 1991.

An Outbreak of Bacteremic *Campylobacter jejuni* Infection

Wayne X. Shandera, M.D., Michael P. Tormey, M.P.H., and Martin J. Blaser, M.D.

Abstract

During September 1980, an outbreak of bacteremic *Campylobacter jejuni* infection occurred in metropolitan Los Angeles. The outbreak was recognized when blood cultures obtained from 11 previously healthy persons with acute febrile illnesses (characterized in over 80% by fever, diarrhea, and headaches) were positive for *C. jejuni*. All recovered after an illness that lasted a mean of 8 days. A surveillance system failed to reveal a concomitant outbreak of gastroenteritis. Isolates had identical biochemical characteristics, susceptibility patterns to antimicrobial agents, and serotypes. Isolates from 2 patients were found to be susceptible to bactericidal activity of normal human serum. When bacteremic case-patients were matched with healthy controls, a significant association ($p < 0.05$, odds ratio 10) between illness and consumption of processed turkey was established. Although turkey was not available for culture, and processing of turkey theoretically destroys *Campylobacter*, turkey carcasses are known to be heavily contaminated with the pathogen.

CAMPYLOBACTER infections in humans typically occur with the species *jejuni, coli,* or *fetus*. *C. jejuni* and *coli* infections produce enteritis, *C. fetus* infections bacteremia. *C. jejuni* and *coli* infections occur in young, healthy adults, while *C. fetus* infections typically affect elderly immunocompromised adults or neonates. The modes of transmission for enteritis in adults with *C. jejuni* are established and include water and foodstuffs, especially undercooked meat products. In this report, we review an outbreak of *C. jejuni* infection that manifested as bacteremia in which a larger outbreak of enteritis was not uncovered; the vehicle appeared to be processed turkey meat.

Description of the Outbreak

During September 1980, an outbreak of bacteremic *Campylobacter* infection occurred in the Los Angeles metropolitan area. The outbreak was recognized when a nurse at a health maintenance organization (HMO) alerted the county health department that within a 4-day period five persons came to a local hospital with an acute, febrile illness; although all received only nonspecific symptomatic therapy, blood cultures had been obtained and the organism *C. jejuni* was isolated from all five; when patients were notified of culture results, four of the five reported a resolution of symptoms in the absence of antimicrobial chemotherapy.

The methods of investigating this outbreak included (a) a review of the HMO laboratory records and a telephone and mail survey of 50 hospital infection-control practitioners to assess the size of the outbreak (this was all performed

---

Supported in part by an interagency agreement between the U.S. Army Medical Research and Development Command and the Veterans Administration.

An earlier version of this paper was given at the Epidemic Intelligence Service Conference, April, 1981, Centers for Disease Control, Atlanta, Georgia 30333.

From the Division of Field Services, Centers for Disease Control Atlanta, Georgia (WXS), Acute Communicable Disease Control, Los Angeles County Department of Health Services, Los Angeles, California (MPT), and Medical Service, Veterans Administration Medical Center, Division of Infectious Diseases, Department of Medicine, University of Colorado School of Medicine, Denver, Colorado (MJB).

Address reprint requests to Wayne X. Shandera, M.D., Department of Internal Medicine, Baylor College of Medicine, Ben Taub Hospital, 2-001, Texas Medical Center, Houston, TX 77030.

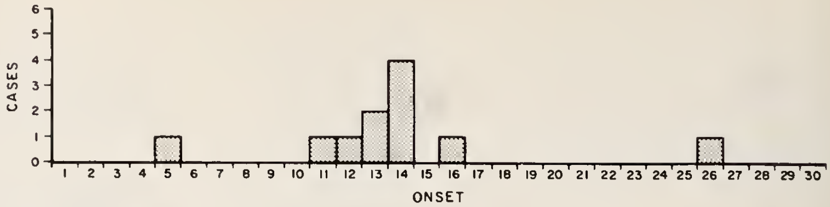


Fig. 1. Dates of isolation of bacteremic *C. jejuni* infections, Los Angeles County, September 1980.

within a month of the outbreak); (b) personal interviews with each of the identified cases and their family members, regarding exposures; (c) Kirby-Bauer (1) antibiotic susceptibility testing; (d) serotyping of 2 available isolates by the Penner system using somatic heat-stable antigens (2, 3); (e) susceptibility testing of the 2 isolates to the bactericidal activity of human serum (4, 5); and (f) a case-control study examining four possible common exposures using a matched-pair analysis with two controls matched per case for age, sex, and neighborhood of residence.

The review of laboratory records and the survey of infection control practitioners demonstrated that during the month of September a total of 11 persons in the Los Angeles metropolitan area sustained an acute febrile illness associated with *C. jejuni* bacteremia. The dates of isolation clustered around September 14 (Fig. 1). Furthermore, the isolation rate of *C. jejuni* from blood cultures at the HMO laboratory increased from a background rate of 0.5 per month (95% confidence intervals, 0.16 to 1.17) during the preceding 32 months to 10 during the month of September, 1980. The number of positive stool cultures during September elicited from the survey was 33, contrasting with 17 during August; additional data on stool samples was not available.

The 11 case-patients who had *C. jejuni* infection during September lived in a range of locales throughout the Los Angeles metropolitan area and included eight males and three females; their mean age was 16 years (range 2 to 32 years) and the mean duration of their symptoms was 8 days. Only two of the cases knew one another (the case on the 26th was a son of a woman who became ill on the 14th, and hence a secondary case) and there were no apparent common exposures.

All 11 had fever (mean maximal temperature was 102.6°F) and 82% developed diarrhea, typically after the onset of fever. Headaches were also reported by 82% and abdominal pain by 73%; abdominal pain, chills, and bloody stools were all reported by more than half (Table 1). Mean pulse rate on examination was 102/min. Data is not available on stool examination (guaiac, white blood cell stain). Laboratory studies included a mean hematocrit of 38.9% (range, 28 to 48.4) and a mean white blood cell count of 7900 (range, 4500 to 16,300). Renal function was unimpaired. One patient was hospitalized for suspect appendicitis. None received antimicrobial therapy.

Isolates were identified at the HMO laboratory using a Bactec system. All isolates had identical sensitivity patterns to antimicrobial agents, including sensitivity to ampicillin, colistin, chloramphenicol, kanamycin, gentamicin, and carbenicillin. Two isolates that were tested according to the Penner system were of the same serotype (one). Both isolates were susceptible to the bactericidal activity of normal human serum ($\log_{10}$ killing 1.37 ± 0.28), similar to the susceptibility of fecal isolates ($\log_{10}$ killing 1.51 ± 0.42) and unlike that of blood stream isolates from previously healthy hosts ($\log_{10}$ killing 0.64 ± 0.32).

Although only 6 of the 11 patients recalled having eaten processed turkey, the case-control study showed that case-patients had eaten turkey more often during the 2 weeks before illness than controls, with an odds ratio of 10.0 ($p < 0.05$) (Table 2). No controls had been ill. No single brand of processed turkey was implicated.

Discussion

This outbreak is distinctive in two respects. First, it represents an outbreak of *C. jejuni* infection in which the primary manifestation of disease was bacteremia. Second, it provides additional epidemiologic support for the role of poultry products in the transmission of *C. jejuni*.

***C. jejuni* Bacteremia.** *C. jejuni* bacteremia is well recognized among immunocompromised patients and among the very young and very old (5–9). However, among previously healthy persons, bacteremia is an infrequent manifestation of *Campylobacter* infections, particularly when contrasted with the large number of fecal isolates that are reported (7). It has been postulated that bacteremia may infrequently follow *C. jejuni* gastroenteritis, since most *C. jejuni* organisms are susceptible to the bactericidal activity of normal human serum (8). However, bacteremia may have been recognized in this outbreak because blood cultures were obtained (transient bacteremia may occur more often than is recognized, since blood cultures are usually not obtained from patients with acute gastroenteritis (9) and because

C. jejuni is not always easy to isolate using blood cultures [10]).

No previous outbreaks of extraintestinal infection among immunocompetent hosts have been described, although an outbreak of *C. jejuni* meningitis has been reported among neonates (11). Since the presentations were primarily a systemic illness and the gastrointestinal symptoms developed secondarily, this outbreak may have been limited to bacteremia. It is possible that the cases of bacteremia represented most readily recognizable infections among an outbreak of predominantly nonbacteremic gastrointestinal illnesses, since stool culture isolations of *C. jejuni* identified through the surveillance system increased during September, and the serum sensitivity of 2 isolates tested were akin to that of typical gastrointestinal isolates; however, no major outbreak of gastrointestinal illnesses was identified at the time of the outbreak though passive or active (telephone calls of emergency rooms) surveillance, and the only data available suggests that the outbreak was one limited to bacteremic infection.

C. jejuni is known to cause frank colitis (10, 12–14) and mucosal invasion (15, 16), although bloodstream infections are uncommon (5) with *C. jejuni,* unlike *C. fetus,* an organism well-known to cause extraintestinal disease (16–18). The reason extraintestinal infections are limited to certain strains is not well understood (4). Both strain characteristics and host factors are responsible for producing *C. jejuni* bacteremia: such infections are more common among strains that are carbohydrate-rich and presumably contain long-chain lipopolysaccharide molecules (8) that confer serum resistance. Such strains are more likely to occur among the immunocompromised, the young, and the elderly (6, 7). Neither strain characteristic or host factors can explain the occurrence of this outbreak. By analogy, *Salmonella* infections often produce bacteremia in previously healthy hosts, and the strain serotype is a major determinant of the propensity toward extra-intestinal spread (19).

Processed Turkey as Source. The case-control study implicated consumption of processed turkey as the common source of the outbreak. Turkey is a highly plausible vehicle for *Campylobacter* transmissions; at slaughterhouses 100% of turkey carcasses are contaminated with *C. jejuni* (20); even after chilling in solution with high chlorine levels, over 80% of turkey carcasses remain culture-positive (21). When turkey wings reach the consumer, more than 50% remain contaminated with *C. jejuni* (22). Similarly, most chicken meat is contaminated, both at the slaughterhouse and when it reaches consumers (21, 23). Handling or consumption of undercooked chicken has been shown to be responsible for epidemic *Campylobacter* infections prior (24) and subsequent (25–27) to the current one, as well as sporadic infections (27–29). In England, an outbreak of enteritis due to *C. jejuni* and *Salmonella hadar* was associated with consumption of a boned, stuffed, and rolled turkey (30).

TABLE 1

Symptoms associated with C. jejuni *infection, Los Angeles County, 1980*

Symptom	Cases	%
Fever	11	100
Diarrhea	9	82
Headache	9	82
Abdominal pain	8	73
Chills	8	73
Bloody stools	6	55
Vomiting	4	36
Arthralgias	4	36
Cough	3	27
Rash	2	18

All controls were asymptomatic.

Why the outbreak resolved in the absence of specific preventive therapy is not known. The actual source of this outbreak could not be traced, but it was postulated that a breakdown in processing occurred. (Processing involves mixing boneless, skinless turkey meat with an emulsion of carcass skins, wing meat, and wing skin; stuffing the mixture into fibrous casings pneumatically and cooking at 180°F for 2–3 hours [31], which should destroy *C. jejuni* present). A breakdown in heating process could have permitted transmission. Consumers typically do not cook this food after purchase, and refrigeration should be associated with a low rate of *C. jejuni* isolation. However, FDA studies demonstrate persistence of *C. jejuni* in processed meat held at 4°C up to 21 days. Viability in several milieus, including poultry, has been shown to be greater when organisms are maintained at 4°C than at 25°C (32, 33).

TABLE 2

Case-Control Study, Bacteremic C. jejuni *Outbreak, Los Angeles County, September 1980, Consumption of Processed Turkey*

	Controls			
Cases	Both ate	1 ate 1 did not	Neither ate	Total
Ate	0	2	4	6
Did not eat	0	1	4	5
Total	0	3	8	11

$\chi^2 = 5.79$ (34)
$p < 0.05$
odds ratio = 10.0

In summary, this article reports an outbreak of bacteremic *C. jejuni* infection in immunocompetent hosts. A larger ourbreak of gastroenteritis, if present, was not readily evident. The serum susceptibility pattern of 2 isolates from the outbreak provided no evidence that unusual virulence was responsible for the bacteremia and infection. Although the actual mechanism of contamination of the food was not determined, a widely distributed product, processed turkey meat, was suggested as the source of the outbreak. The improved control and prevention of *C. jejuni* infections will require the continued investigation into its epidemiology and pathogenesis.

Acknowledgments

The authors thank Phyllis Fliegel, R.N., and William Weinstein, M.D., for collection of data; John Penner, Ph.D., for serotyping the strains; and Frank Sorvillo, M.P.H., Paul Blake, M.D., M.P.H., and Robert Gunn, M.D., M.P.H.

References

1. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45:493–496.
2. Penner JL, Hennessy JN. Passive hemagglutination technique for serotyping *Campylobacter fetus* subspecies *jejuni* on the basis of soluble heat-stable antigens. J Clin Microbiol 1980; 12:732–737.
3. Lastovica AJ, Penner JL. Serotypes of *Campylobacter jejuni* and *Campylobacter coli* in bacteremic, hospitalized children. J Infect Dis 1983; 147:592.
4. Blaser MJ. Extraintestinal *Campylobacter* infections. West J Med 1986; 144:353–354.
5. Blaser MJ, Perez GP, Smith PF, Patton C, Tenover FC, Lastovica AJ, Wang WI. Extraintestinal *Campylobacter jejuni* and *Campylobacter coli* infections: host factors and strain characteristics. J Infect Dis 1986; 153:552–559.
6. Dhawan VK, Ulmer DD, Nachum R, Rao B, See RC. *Campylobacter jejuni* septicemia—epidemiology, clinical features and outcome. West J Med 1986; 144:324–328.
7. Riley LW, Finch MJ. Results of the first year of national surveillance of *Campylobacter* infections in the United States. J Infect Dis 1985; 151:956–959.
8. Blaser MJ, Smith PF, Kohler PF. Susceptibility of *Campylobacter* isolates to the bactericidal activity in normal serum. J Infect Dis 1985; 151:227–235.
9. Blaser MJ, Reller LB, Luechtefeld NW, Wang WL. *Campylobacter* enteritis in Denver. West J Med 1982; 136:287–290.
10. Wang WL, Blaser MJ. Detection of pathogenic *Campylobacter* species in blood culture systems. J Clin Microbiol 1986; 23:709–714.
11. Goossens H, Henocque G, Kremp L, Rocque J, Boury R, Alanio G, Vlaes L, Hemelhof W, van den Borre C, Macart M, Henocque G, Butzler JP. Nosocomial outbreak of *Campylobacter jejuni* meningitis in newborn infants. Lancet 1986; 2:146–149.
12. Drake AA, Gilchrist MJ, Washington JA, Huizenga KA, van Scoy RE. Diarrhea due to *Campylobacter fetus* subspecies *jejuni:* A clinical review of 63 cases. Mayo Clin Proc 1981; 56:414–423.
13. Skirrow MB, *Campylobacter* enteritis: A "new" disease. Br Med J [Clin Res] 1977; 2:9–11.
14. Lambert ME, Schofield PF, Ironside AG, Mandal BK. *Campylobacter* colitis. Br Med J [Clin Res] 1979; 1:857–859.
15. Duffy MC, Benson JB, Rubin SJ. Mucosal invasion in *Campylobacter* enteritis. Am J Clin Pathol 1980; 73: 706–708.
16. Butzler JP, Skirrow MB. *Campylobacter* enteritis. Clin Gastroenterol 1979; 8:737–765.
17. Blaser MJ, Reller LB. *Campylobacter* enteritis. N Engl J Med 1981; 305:1444–1452.
18. Guerrant RL, Lahita RG, Winn WC, Roberts RB. Campylobacteriosis in man: pathogenic mechanisms and review of 91 bloodstream infections. Am J Med 1978; 65:584–592.
19. Blaser MJ, Feldman RA. *Salmonella* bacteremia: reports to the Centers for Disease Control, 1968–1979. J Infect Dis 1981; 143:743–746.
20. Luechtefeld NW, Wang WL. *Campylobacter fetus* subsp. *jejuni* in a turkey processing plant. J Clin Microbiol 1981; 13:266–268.
21. Simmons NA, Gibbs FJ. *Campylobacter* subspp. in oven-ready poultry. J Infect 1979; 1:159–162.
22. Rayes HM, Genigeorgis CA, Farver TB. Prevalence of *Campylobacter jejuni* on turkey wings at supermarket level. J Food Protect 1983; 46:292–294.
23. Blaser MJ, Taylor DN, Feldman RA. Epidemiology of *Campylobacter jejuni* infections. Epidemiol Rev 1983; 5:157–176.
24. Brouwer R, Mertens MJA, Siem TH, Katchaki J. An explosive outbreak of *Campylobacter* enteritis in soldiers. Antonie Van Leeuwenhoek 1979; 45:517–519.
25. Istre GR, Blaser MJ, Shillam P, Hopkins RS. *Campylobacter* enteritis associated with undercooked barbecue chicken. Am J Public Health 1984; 74:1265–1267.
26. Rosenfeld JA, Arnold GJ, Davey GR, Archer RS, Woods WH. Serotyping of *Campylobacter jejuni* from an outbreak of enteritis implicating chicken. J Infect 1985; 11:159–165.
27. Tauxe RV, Pegues DA, Hargrett-Bean N. *Campylobacter* infections: the emerging national pattern. Am J Public Health 1987; 77:1219–1221.
28. Hopkins RS, Scott AS. Handling raw chicken as a source of sporadic *Campylobacter jejuni* infections. J Infect Dis 1983; 148:770.
29. Oosterom J, den Uyl C, Banffer JR, Huisman J. Epidemiological investigations on *Campylobacter jejuni* in households with a primary infection. J Hyg (Lond) 1984; 93:325–332.
30. Communicable Disease Surveillance Center. Surveillance of food poisoning and salmonellosis. Communicable Disease Report 1981; 26:5.
31. Kramlich WE, Pearson AM, Tauber FW. Meat cookery and cooked meat products. In: Kramlich WE, Pearson AM, eds. Processed meats. Westport CT: Avi Publishing Company, 1984:96–107.
32. Blaser MJ, Hardesty HL, Powers B, Wang WL. Survival of *Campylobacter fetus* subspecies *jejuni* in biological milieus. J Clin Microbiol 1980; 11:309–313.
33. Blankenship LC, Craven SE. *Campylobacter jejuni* survival in chicken meat as a function of temperature. Appl Environ Microbiol 1982; 44:88–92.
34. Mantel N, Haenzel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22:719–748.

Submitted for publication February 1990.
Final revision received July 1991.

Acute Myocardial Infarction in a Long-Term Care Institution for the Aged

IVONNE Z. JIMENEZ-VELAZQUEZ, M.D., MANUEL RODSTEIN, M.D., MICHAEL MULVIHILL, DR.P.H.,
MING-ANN HSU, M.P.H., LESLIE S. LIBOW, M.D., AND RICHARD R. NEUFELD, M.D.

Abstract

We reviewed the clinical characteristics and outcome of cases of acute myocardial infarction occurring from January 1, 1985, through December 31, 1987, in the population of a long-term care institution for the elderly. The total number of patients in the series was 43. Comparisons were made between those patients transferred to a general acute-care hospital and those who remained at the facility. The most common initial symptoms of acute myocardial infarction in 32 of 48 patients were, in order, dyspnea, dizziness or syncope, precordial pain, and abdominal pain. Nine (of 43) patients were asymptomatic. In the 14 (of 43) patients transferred to an acute-care hospital, cardiac failure, arrhythmias, and cardiogenic shock were much more frequent than among those retained in the long-term care facility. We concluded that a high index of suspicion for the diagnosis of acute myocardial infarction in the institutionalized elderly is indicated. Patients with mild infarction can be retained in long-term care institutions; resulting mortality from cardiac disorders should be low in adequately staffed and equipped long-term care institutions.

MYOCARDIAL infarction is a major cause of morbidity and mortality in patients in long-term care facilities (1). The signs and symptoms of infarction in elderly persons differ from those in younger individuals (2).

This study was undertaken to review the clinical characteristics and outcome of cases of acute myocardial infarction occurring over a three-year period in the population of a long-term care institution for the elderly. Comparisons were made between those patients transferred to a general acute-care hospital and those who remained at the facility.

Materials and Methods

We reviewed the records of 43 patients at a long-term care institution, The Jewish Home and Hospital for Aged, who were diagnosed as undergoing acute myocardial infarction during the period January 1, 1985 through December 31, 1987.

Definitions. The electrocardiographic diagnosis of acute myocardial infarction was made on the discovery of acute electrocardiographic changes, including new significant Q waves, S-T segment elevations or depressions, T-wave inversions, and loss of amplitude or alteration of R waves, with characteristic evolution over weeks or months.

The criteria for the diagnosis of acute myocardial infarction were those of the Minnesota Code (3) and the Multicenter Post-Infarction Research Group (4). Notation was made of isoenzyme levels, fever, sedimentation rate, cardiac and pulmonary auscultatory findings, chest x-ray, and evidence of failure and cardiogenic shock at the time of diagnosis of acute myocardial infarction.

Transfer to other institutions was noted, and

Presented at the American Geriatrics Society Meeting in Anaheim, CA, June 1988, and The New York Academy of Medicine, March 1989.

From The Jewish Home and Hospital for Aged, Medical Department, New York, NY, and the Mount Sinai School of Medicine, Department of Geriatrics and Adult Development, One Gustave Levy Place, New York, NY. Address reprint requests to Manuel Rodstein, M.D., Director of Cardiology, The Jewish Home and Hospital for Aged, Kingsbridge Division, 100 West Kingsbridge Road, Bronx, NY 10468.

TABLE 1
Age Characteristics by Sex, 43 Patients with Acute Myocardial Infarction

	Male (N = 14)	Female (N = 29)
Age (mean ± SD)*	81.6 ± 7.0	87.4 ± 6.2
Age (years)		
70–79	5 (35.7)	2 (6.9)
80–89	6 (42.9)	16 (55.2)
90+	3 (21.4)	11 (37.9)

* $p < .01$ by *t*-test.

mortality within 30 days of diagnosis was tracked.

Results

In the total population of the long-term care institution, the male:female ratio was 1:4; but in the 43 patients in this study the male:female ratio was 1:2. The men in the study were significantly younger (average age) than the women (Table 1). The initial symptoms in order of frequency are recorded in Table 2. The frequency of symptoms among those transferred to the acute-care hospital was significantly greater than those remaining at the long-term care institution ($p <$ .05). Of the 9 patients who were completely asymptomatic, 2 were diagnosed at admission, 5 at annual examination, one at clearance for surgery, and one in the follow-up electrocardiogram one week after the onset of atrial fibrillation. Only 2 of the 13 patients with significant mental impairment recorded precordial pain.

Isoenzyme levels were elevated in 13 of the 38 patients so tested at the long-term care institution. Other clinical and laboratory findings are detailed in Table 3.

The decision to transfer a patient was found to be based on the presence and severity of congestive heart failure and arrhythmias. Those transferred to the acute-care hospital tended to be slightly younger.

The overall mortality in the 30-day period following the diagnosis of acute myocardial infarction was similar for patients transferred from and remaining at the long-term care institution, despite the greater severity of disease in the transferred group, because deaths from noncardiac causes were higher in the group not transferred (Table 4).

Discussion

Kannel and Abbot (5) reported on the Framingham study based on the periodic evaluation of an ambulatory population that included both recognized and unrecognized myocardial infarctions. Twenty-three percent of infarctions occurring in persons between 65 and 74 years of age, and 34% of infarctions in persons between 75 and 84 years of age, were unrecognized. In the reported total of 708 cases of unrecognized myocardial infarctions in persons of all ages, more than 25% were discovered solely on the appearance of new diagnostic evidence in routine biennial electrocardiograms. About half of these were completely silent—asymptomatic—and half had atypical symptoms. The unrecognized infarctions were more common in those over 60 years of age and among women.

Kannel (6) feels that the data underestimated unrecognized myocardial infarction, since

TABLE 2
Initial Symptoms and Mental Status of 43 Patients with Acute Myocardial Infarction by Treatment Site

	Remained at JHHA (N = 29)		Transferred to MSH (N = 14)		Total (N = 43)	
Characteristic	N†	%	N†	%	N†	%
Initial symptom*						
Dyspnea	9	31.0	9	64.3	18	41.9
Precordial pain	4	13.8	6	42.8	10	23.3
Dizziness or syncope	7	24.1	5	35.7	12	27.9
Diaphoresis	5	17.2	6	42.8	11	25.6
Abdominal symptoms	4	13.8	5	35.7	9	20.9
Other noncardiac pain	4	13.8	5	35.7	9	20.9
No symptoms*	9	31.0	0	0.0	9	20.9
Mental Status Impairment						
None or mild	18	62.1	12	85.7	30	69.8
Moderate or severe	11	37.9	2	14.3	12	30.2

* Significant at $p < 0.05$ by Fisher's exact test.
† Total exceeds 100% because some patients had more than one symptom.

those patients who died due to acute myocardial infarction out of the hospital or whose electrocardiograms reverted to nondiagnostic patterns were not included.

A previous study of 50 patients by Rodstein (2) detailed the frequency of precordial pain and dyspnea as well as complete absence of symptoms in those patients who were diagnosed as having acute myocardial infarction or who had characteristic changes in a routine electrocardiagram.

Pathy (7) in a series of 387 hospital admissions of persons over the age of 64 found precordial pain in 19.4%, dyspnea in 19.6%, and cerebrovascular symptoms, including confusion, syncope, stroke, giddiness, vertigo, and faintness, in 42.6%. Our findings are comparable.

On the other hand, Applegate (8) and Tinker (9) reported precordial pain in 61.8% and 58.6% respectively in groups averaging 75.6 and 73.8 years of age.

Despite the differences in age and site of care, the overall mortality of 30% in our series of patients is quite similar to those of patients over 75 years of age admitted to hospitals. Such mortality rates have ranged from 25.3% to 40% (10–14). The exception is the series of patients over the age of 70 admitted to an intensive coronary care unit reported by Berman (15), in which mortality was 16.4%.

Comparison of the group of patients cared for at The Jewish Home and Hospital for Aged with the group of patients transferred revealed that those transferred had more frequent and intense precordial pain, dyspnea, and other pain as well as statistically significant increases in heart failure and cardiogenic shock. The transferred patients were somewhat younger. None of the asymptomatic patients were transferred. Those with more severe degrees of mental impairment tended to be retained at The Jewish Home and Hospital for Aged.

The failure to observe elevated isoenzyme levels in the majority of the 43 patients studied may be attributed to several factors. Most important, blood was not drawn from several patients prior to their emergency transfer to the hospital, and the hospital records were not obtained for this study. A second reason was delay in measuring isoenzyme levels in those asymptomatic patients undergoing diagnostic electrocardiograms as part of a routine annual examination and in patients who had atypical symptoms of infarction, such as with senile dementia.

TABLE 3

Physical and Laboratory Findings at Onset of Acute Myocardial Infarction (N = 43)

Finding	# Positive/ # performed	% Positive
High isoenzyme level	13/38	34.2
Leukocytosis	21/34	61.8
Fever	14/38	36.8
Diaphoresis	11/43	25.6

TABLE 4

Outcome of Acute Myocardial Infarction by Treatment Site

	Remained at JHHA (N = 29)		Transferred to MSH (N = 14)	
Outcome	N	%	N	%
Cardiac death*	4*	13.8	5	35.7
Noncardiac death	4	13.8	0	—
Recovery	21	72.4	9	64.3
Overall mortality†	8	27.6	5	35.7

* One cardiac death was a death on arrival at the hospital.

† Deaths within 30 days after discovery of infarction.

In summary, atypical presentations predominated in a group of 43 aged patients in a long-term care facility who sustained an acute myocardial infarction. Asymptomatic patients were not uncommon. Precordial and other types of pain were less frequent than dyspnea and cerebrovascular symptoms.

These findings indicate the wisdom of suspecting acute myocardial infarction in the institutionalized aged. Those patients with few major symptoms or signs who were retained for care in the institution experienced a lower immediate mortality rate due to cardiac causes than those who required emergency transfer to an acute-care hospital, but the overall mortality rate in the 30 days following diagnosis was similar in transferred and nontransferred patients.

Acknowledgments

We wish to thank Mrs. Frances Feltz and the Medical Records staff for their assistance in the process of record review and Ms. Neomia Cook for her technical assistance.

References

1. Rossman I, Rodstein M, Bornstein M. Undiagnosed diseases in an aging population. Arch Intern Med 1974; 133:366–369.
2. Rodstein M. The characteristics of non-fatal myocardial infarction in the aged. Arch Intern Med 1956; 98:84–90.
3. Blackburn H, Keys A, Simonson E, et al. The electrocardiogram in population studies: A classification system. Circulation 1960; 21:1160.
4. Greenberg H, Gillespie J, Dwyer EM Jr., and the Multicenter Post-Infarction Research Group. A new electrocardiographic classification for post-myocardial infarction clinical trials. Am J Cardiol 1987; 58:1057–1063.
5. Kannel WB, Abbot RD. Incidence and prognosis of unrec-

ognized myocardial infarction. N Engl J Med 1984; 311:1114–1147.

6. Kannel WB. Prevalence and clinical aspects of unrecognized myocardial infarction and sudden unexpected death. Circulation 1987; 75(Part II #3):4–5.
7. Pathy MS. Clinical presentation of myocardial infarction in the elderly. Br Heart J 1967; 29:190–199.
8. Applegate WB, Graves S, Collins T, Vanderzwaag R, Akins D. Acute myocardial infarction in elderly patients. South Med J 1984; 77:1127–1129.
9. Tinker GM. Clinical presentation of myocardial infarction in the elderly. Age Ageing 1981; 10:237–240.
10. Sagie A, Rotenberg Z, Weinberger I, Fuchs J, Agnon J. Acute transmural myocardial infarction in elderly patients hospitalized in the coronary care unit versus the general medical ward. J Am Geriatr Soc 1987; 35:915–919.
11. Latting CA, Silverman M. Acute myocardial infarction in hospitalized patients over age 70. Am Heart J 1980; 100:311–318.
12. Chatuverdi NC, Shivalingappa G, Shanks B, et al. Myocardial infarction in the elderly. Lancet 1972; 1:280–281.
13. Kincaid D, Botti RE. Acute myocardial infarction in the elderly. Chest 1973; 64:170–172.
14. Williams BO, Begg TB, Semple T, et al. The elderly in a coronary care unit. Br Med J 1976; 2:451–453.
15. Berman ND. The elderly patient in the coronary care unit. J Am Geriatr Soc 1979; 27:145–151.

Submitted for publication October 1990.
Final revision received November 1991.

Screening Indices for Cytomegalovirus Retinitis in Patients with Human Immunodeficiency Virus

Gregory A. Butler, B.A., and Alan H. Friedman, M.D.

Abstract

Cytomegalovirus retinitis, the major cause of blindness in patients with the acquired immunodeficiency syndrome, can be arrested by early detection and treatment. We identified 9 screening indices for early CMV retinitis: T-4/T-8 ratio < 0.11, T-4 count < 30, T-8 count < 500, leukocytes $< 4,200$, platelets $< 240,000$, hemoglobin < 11.6, hematocrit < 35, <6 for a diagnostic profile which incorporates these 7 indices, and <2 for the IA-sum which compounds T-cell inversion and anemia. A threshold value was determined for each index, identified by retrospective review of charts of 15 patients with CMV retinitis and 30 without. Sensitivity, specificity, positive predictive value, negative predictive value, and receiver operating characteristic curve area was calculated. The best screening index was the T-cell ratio, which had the highest sensitivity (0.93), negative predictive value (0.94–0.99) and receiver operating characteristic curve area (0.89), and a positive predictive value among the highest (0.50–0.82).

CYTOMEGALOVIRUS (CMV) retinitis is the most common cause of vision loss in patients with the acquired immunodeficiency syndrome (AIDS), among whom it has a reported incidence of 15% to 45% (1–7). Extensive retinal damage may occur before symptoms appear (1, 2, 5, 6), progressing to blindness and loss of independence (8). Drug therapy for symptomatic patients may prevent further retinal necrosis, but recovery of vision is restricted to the small improvement resulting from resorption of edema (1, 2, 5, 6).

HIV-seropositive patients are generally more susceptible to opportunistic infections as their physical condition worsens (9–11). Nonspecific, prophylactic treatment for CMV retinitis is inadvisable, however, since currently available drugs are expensive, may be nephrotoxic or produce neutropenia with prolonged use, and require maintenance of a central venous line for daily administration (12, 13).

CMV retinitis is diagnosed by indirect ophthalmoscopy (2, 5, 14). Examination of all HIV-seropositive patients at the frequency needed to preclude interexamination progression of the retinitis would be prohibitively expensive while augmenting patients' anxiety. The high rate of negative examinations would lessen compliance with referrals for ophthalmoscopy, which is already less than 50% at The Mount Sinai Hospital. This article describes a study conducted to explore the utility of various physiologic and sociodemographic factors as indices of CMV retinitis, so that asymptomatic patients at high risk of developing the disease might be identified and cogently advised to have regular examinations.

Methods

Subjects. This study included 45 patients of the Mount Sinai AIDS Clinic, aged 20–50, who were HIV seropositive by Western blot. Fifteen

---

Submitted as partial fulfillment of the clerkship requirement in Community Medicine while the first author was a third year student at Mount Sinai School of Medicine, and approved by the institutional review board. The second author is a Professor in the Department of Ophthalmology, Mount Sinai School of Medicine, and acted as field preceptor during the clerkship and as advisor during the writing of this report. Address reprint requests to Dr. Alan Friedman, Department of Ophthalmology, Box 1183, Mount Sinai School of Medicine, One Gustave Levy Place, New York, NY 10029.

TABLE 1
Threshold Values of Indices for CMV Retinitis

Index	Value
IA-Sum	<2
T-4/T-8 ratio	<0.11
Diagnostic Profile	<6
T-4 count (cu mm)	<30
Hemoglobin (g/dL)	<11.6
Hematocrit (%)	<35
Platelets (×1000/µL)	<240
T-8 count (cu mm)	<500
Leukocytes (×1000/µL)	<4.2

had been diagnosed as having CMV retinitis by indirect ophthalmoscopy (cases) and 30 had not been diagnosed as having the disease (controls/companion group).

Cases. This group consisted of 15 patients of the Mount Sinai AIDS Clinic with CMV retinitis diagnosed between January 1990 and April 1991. (Patients of the AIDS Clinic are referred for indirect ophthalmoscopy as a routine component of their initial evaluation and upon presenting with visual symptoms. About 50% of those subjects having no visual symptoms and 100% of those having visual symptoms complied with referrals for ophthalmologic exam.) Ten cases were still being followed in the Clinic at the time of this study, April 1991, and were identified by the nurses and social workers there. The remaining 5 cases had died in the period from February 1990 to January 1991. They were identified during the chart review described below. Data analysed for cases in this study were recorded within 2 months of diagnosis of CMV retinitis.

TABLE 2
Accuracy of Indices for CMV Retinitis

Index	Sensitivity	Specificity	Predictive value*	
			Positive	Negative
IA-Sum	0.71	0.89	0.55–0.84	0.75–0.76
T-4/T-8 ratio	0.93	0.84	0.50–0.82	0.94–0.99
Diagnostic Profile	0.64	0.88	0.50–0.81	0.71–0.72
T-4 count	0.83	0.59	0.26–0.62	0.80–0.94
Hemoglobin	0.73	0.55	0.22–0.56	0.71–0.92
Hematocrit	0.80	0.50	0.22–0.46	0.73–0.76
Platelets	0.79	0.45	0.20–0.54	0.74–0.93
T-8 count	0.91	0.31	0.19–0.52	0.81–0.96
Leukocytes	0.80	0.37	0.18–0.51	0.67–0.69

* Limits of positive and negative predictive value were calculated using prevalence rates reported in the literature (0.15–0.45).

Companion Group. Charts of 125 patients of the Mount Sinai AIDS Clinic who had died of AIDS between February 1990 and January 1991 were requested from Medical Records. Using charts of dead patients for the companion group and 5 of 15 cases made available a greater number of subjects for this study.

Seventy-five percent of the companion group had negative retinal examinations subsequent to the dates of data used for controls. None of the remaining 25% had visual symptoms for the duration of their care at the Clinic, that is, for 2–6 months after the date of data used for each subject; thus it is less likely that they had CMV retinitis at the time of serum collection. Data used

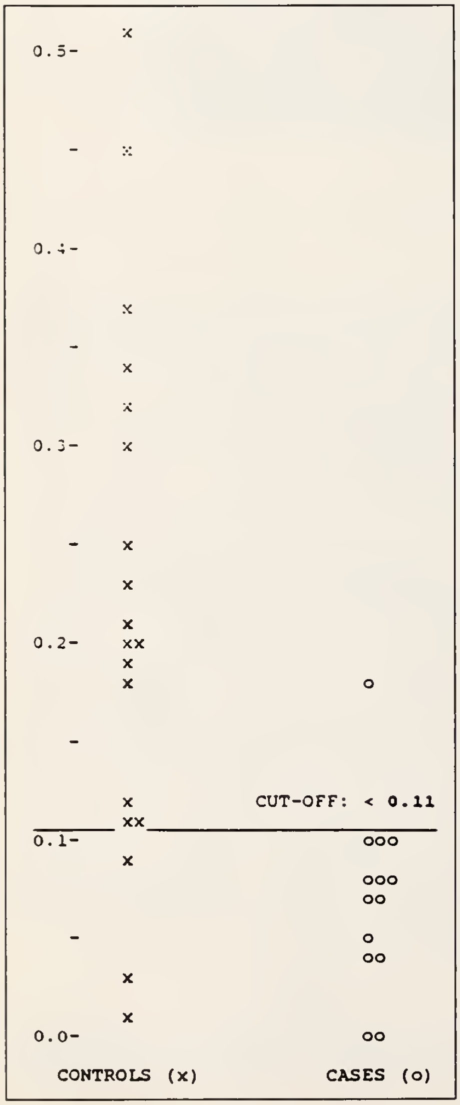


Fig. 1. Threshold determination for T-4/T-8 ratio. Ratio for subjects having CMV retinitis was plotted in linearly scaled column, and ratio for each companion subject was plotted in a parallel. Threshold ratio was identified as that which best discriminated between cases and controls.

TABLE 3
Area Under Received Operating Characteristic Curve for Each Index

Index	Area
T-4/T-8 ratio	0.89
IA-Sum	0.83
Diagnostic Profile	0.77
T-4 count	0.74
Leukocytes	0.66
Hemoglobin	0.62
Hematocrit	0.62
Platelets	0.61
T-8 count	0.53

for the companion group were from dates within a month of their initial evaluation by the Clinic.

Eighty-five charts were made available, and 41 of these had the minimal data required for this study, including SMAC, age, gender, infection risk, ethnicity, and notes on their clinical course. T-cell data were available for 14 of the 15 cases and 19 of the 30 controls. Five charts were found to be those of patients with CMV retinitis and were added to the group of cases. Two charts were those of infants, and another was for a patient whose age was above the range of 20–50 years chosen as representative of most AIDS Clinic patients. The data in charts for a final 4 patients was found to be for dates too late in the course of AIDS to be representative of patients when they typically first present at the AIDS Clinic. These 7 charts were excluded from review, and the remaining 30 charts were assigned to the companion group.

The yield of charts useful for this study was confirmed by Medical Records personnel to be usual for large requests; no systematic bias was evident in their selection.

Diagnosis of CMV Retinitis. The cases in this study (true positives and false negatives) were diagnosed as having CMV retinitis based on observation of the characteristic "ketchup and cheese" lesion on indirect ophthalmoscopy (2, 5, 14, 15) by the Mount Sinai ophthalmologic pathologist.

Identification of Indices. Potential markers studied included factors found to be prognostically significant in other studies on AIDS patients—anemia, neutropenia, coinfections, the T-4 count (9–11)—as well as the T-4/T-8 ratio, the T-8 count, liver enzymes, albumin, infection risk factors, and ethnicity. The distributions of 7 physiological factors, T-cell ratio, T-4 number, T-8 number, leukocyte and platelet counts, hemoglobin concentration, and hematocrit differed between the two groups of patients. For each of these, a cut-off point was identified that best discriminated between CMV retinitis patients and the companion group. (Table 1, Fig. 1).

Diagnostic Profile. The utility of a compound index, the Diagnostic Profile, encompassing all 7 factors identified above was explored. One point

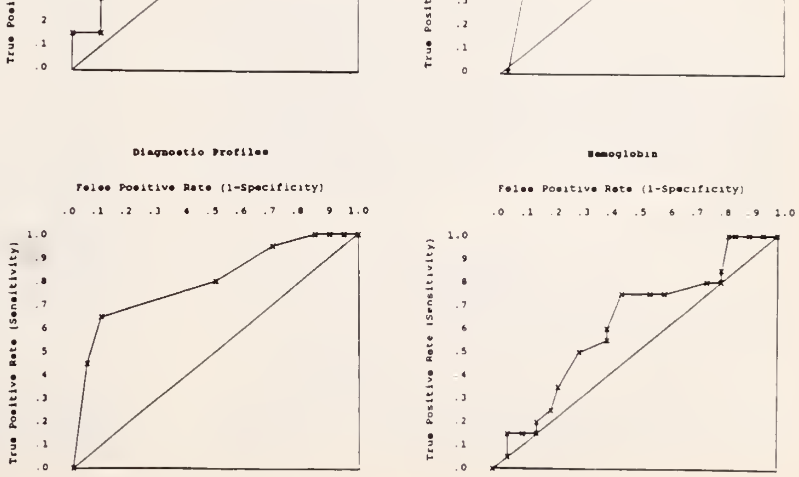


Fig. 2. Receiver operating characteristic curves for the three indices having the greatest areas under the curve and correspondingly highest positive predictive values, the T-4/T-8 ratio, the I-A Sum, and the Diagnostic Profile, and for hemoglobin concentration, an index specific for anemia. Areas under curves were determined by trapezoid method.

was awarded for a value below the cut-off point for each of the 7 indices listed in the previous section and no points for values equal to or above the cut-off, for a total of 0–7 points. A cut-off for the Diagnostic Profile was identified which discriminated best between cases and controls.

IA-Sum. This index combined T-cell inversion and anemia and was chosen as a simpler means than the Diagnostic Profile to reflect the pathophysiologic processes inferred from our study to contribute to HIV-seropositive patients developing CMV retinitis. A T-cell ratio less than 0.11 and a hemoglobin concentration less than 11.6 g/dL were each awarded 1 point, and no points were awarded for data values equal to or above the threshold, for a total of 0–2 points. A cut-off point was identified which best discriminated between cases and controls. Hemoglobin concentration was used rather than hematocrit because hemoglobin concentration has the higher positive predictive value (PPV). Gender-specific hemoglobin cut-off points, which might have been desirable to account for the different distributions of concentrations normal for males and females, were precluded by the small number of cases.

Statistical Analysis. A true positive result was defined as occurring when a patient ophthalmologically diagnosed with CMV retinitis was selected by an indicator to have the disease, a false positive when CMV retinitis was indicated in a patient found to be negative on ophthalmologic examination or having no visual symptoms for at least 3 months after the date of data collection. A false negative occurred when a patient ophthalmologically diagnosed as having CMV retinitis was indicated not to have the disease. Sensitivity was calculated as

$$(\text{true positives})/(\text{true positives} + \text{false negatives})$$

and specificity as

$$(\text{true negatives})/(\text{true negatives} + \text{false positives}).$$

The prevalence of CMV retinitis among the 45 patients of this study (0.33) was artifactual by design. Thus we used the prevalence reported in the literature, 0.15–0.45 (1–7), to calculate the upper and lower limits of positive predictive values as

$$(\text{sensitivity} \times \text{prevalence})/([\text{sensitivity} \times \text{prevalence}] + [(1 - \text{specificity}) \times (1 - \text{prevalence})])$$

and of negative predictive value (16) as

$$(\text{specificity} \times [1 - \text{prevalence}])/([\text{specificity} \times \text{prevalence}] + [(1 - \text{sensitivity}) \times \text{prevalence}])$$

Area under the receiver operating characteristic curve was calculated nonparametrically by the trapezoid rule (17).

Results

For each screening index, the value which discriminated best between patients with CMV retinitis and other HIV-seropositive patients (Table 1), the derivations of selected thresholds (Fig. 1), and the accuracy of each index (Table 2) were calculated. Sensitivity was highest for the T-4/T-8 ratio (0.93) and the T-8 count (0.91), and lowest for the Diagnostic Profile (0.64). Specificity was highest for the IA-Sum (0.89) and the Diagnostic Profile (0.88) and lowest for the T-8 count (0.31). Positive predictive value was highest for the IA-Sum (0.55–0.84), the T-4/T-8 ratio (0.5–0.82) and the Diagnostic Profile (0.50–0.76), and considerably lower for the other indices (<0.26–0.62). Negative predictive value was highest for the T-4/T-8 ratio (0.94–0.99), and lowest for the leukocytes (0.67–0.69).

The areas under the ROC curves (Table 3) and the curves for selected indices (Fig. 2) were plotted. The areas for all 8 indices were larger than that of a test having a discriminatory ability no better than chance (0.5). The areas were greatest for the T-4/T-8 ratio (0.89), the IA-Sum (0.83), and the Diagnostic Profile (0.77), and smallest for the T-8 count (0.53).

Discussion

The best screening index for CMV retinitis was the T-4/T-8 ratio, which had the highest sensitivity, negative predictive value, and area under the receiver operator curve, and a positive predictive value surpassed only by the IA-Sum. These results support the findings of Gross et al. (1), Justice et al. (9), Wenger (10), and others that the opportunistic infections of HIV-seropositive patients, including CMV retinitis, are accompanied by a generalized depression of immunity-conferring cells and functional components of blood below a critical level. Thus, it might be assumed that all patients having low T-cell counts would be at high risk for CMV retinitis. However, it is desirable to have an index with a high predictive value which could be used to screen for the disease to improve patient compliance with referrals for ophthalmologic examinations and the applicability and thoroughness of each examination, while minimizing patient anxiety.

The Diagnostic Profile and IA-Sum were derived to reflect the cumulative pathophysiologic effect of T-cell inversion, anemia, and cytopenia, and thus were expected to have greater positive predictive values than any component. The IA-Sum had the highest predictive value (0.55–0.84) of all the indices, but the T-4/T-8 ratio (0.50–0.82) had comparable predictive value, and its considerably higher sensitivity (0.93 versus 0.71) made it better suited to screening.

Receiver operating characteristic (ROC) analysis provides a description of an index's discriminatory value independent of both disease prevalence and decision threshold (18). The trapezoid method used in this study underestimates the area under the ROC curve to a greater extent than more parametric methods; nevertheless, it permits comparison between the area for each index and the area for a nondiscriminatory test (0.5). The area under the ROC curve for the T-4/T-8 ratio (0.89) compares favorably with that of indices used in the diagnosis of other diseases, for example the ratio of forced expiratory volume in one second to forced vital capacity, which is used in diagnosing obstructive lung disease (0.86), and acid phosphatase levels, which are used to diagnose prostatic carcinoma (0.75–0.80) (19, 20).

The T-4/T8 ratio provides a simple, noninvasive, inexpensive means of accurately identifying patients at higher risk for CMV retinitis and of motivating them to comply with referrals for regular indirect ophthalmoscopy, permitting early detection and treatment of more patients with CMV retinitis.

Data on this "development" set of 45 subjects permitted determination of values of each index studied which best differentiated patients diagnosed with CMV retinitis from those without the disease. A prospective "validation" study would permit a challenge of these threshold values so that any differences between the subjects in this study and other HIV-seropositive patients might be accounted for. The expanded time frame of such a study would permit inclusion of a larger number of subjects and exclusion of those patients not compliant with ophthalmologic examination at the time data were to be collected.

Acknowledgments

We are indebted to Ellen Fischer of the Mount Sinai Medical Center Department of Epidemiology and Community Medicine for her advice on data analysis; Collette Severin of the Mount Sinai Medical Center Department of Ophthalmology; and Drs. David Rose and Tony Cheung and nurses William Davis and Edna Alcantara for their assistance at the AIDS Clinic.

References

1. Gross JG, Bozzette SA, Matthews WC, et al. Longitudinal study of cytomegalovirus retinitis in acquired immunodeficiency syndrome. Ophthalmology 1990; 97:681–686.
2. Bloom JN, Palestine AG. The diagnosis of cytomegalovirus retinitis. Ann Int Med 1988; 109:963–969.
3. Holland G, Prepose J, Pettite T, et al. Acquired immunodeficiency syndrome: ocular manifestations. Ophthalmology 1983; 90:859–872.
4. Holland G, Sidikaro Y, Krieger A, et al. Treatment of cytomegalovirus retinopathy with gancyclovir. Ophthalmology 1987; 815–823.
5. Hennis HL, Scott AA, Apple DJ. Cytomegalovirus. Surv Ophthalmol 1989; 34:193–203.
6. Palestine AG. Clinical aspects of cytomegalovirus retinitis. Rev Infect Dis 1988; 10:S515–531.
7. Schmitt-Graff A, Neuen-Jacob E, Rettig B, Sundmacher R. Evidence for cytomegalovirus and human immunodeficiency virus infection of the retina in AIDS. Virchows Arch 1990; 416:249–253.
8. Schuman JS, Orellana J, Friedman AH, Teich SA. Acquired immunodeficiency syndrome. Surv Ophthalmol 1987;31:384–409.
9. Justice AC, Feinstein AR, Wells CK. A new prognostic system for the acquired immunodeficiency syndrome. N Engl J Med 1989; 320:1388–1393.
10. Wenger JD, Whalen CC, Lederman MM, Spech TJ, Carey JT, Tomford JW, Landefeld CS. Prognostic factors in acquired immunodeficiency syndrome. J Gen Int Med 1988; 3:464–469.
11. Siegal F, Fitzgerald P, et al. Opportunistic infections in acquired immunodeficiency syndrome result from synergistic defects of both the natural and adaptive components of cellular immunity. J Clin Invest 1986; 78:115–123.
12. Orellana J, Teich S, Friedman A, et al. Combined short- and long-term therapy for the treatment of cytomegalovirus retinitis using gancyclovir. Ophthalmology 1987; 94:831–838.
13. Millar AB, Miller RF, Patou G, Mindel A, Marsh R, Semple SJG. Treatment of cytomegalovirus retinitis with zidovudine and gancyclovir in patients with AIDS: outcome and toxicity. Genitourinary Med 1990; 66:156–158.
14. Friedman AH. The retinal lesions of the acquired immunodeficiency syndrome. Trans Am Ophthalmol Soc 1984; 82:447–491.
15. Henderly DE, Freedman WR, Smith RE, Causey D, Rao NA. Cytomegalovirus as the initial manifestation of the acquired immunodeficiency syndrome. Am J Ophthalmol 1987; 103:316–320.
16. Fletcher RH, Fletcher SW, Wagner EH. Clinical epidemiology, 2nd ed. Baltimore: Williams & Wilkins, 1988, 56.
17. Hanely JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 1983; 148:839–843.
18. Metz CE. Basic principle of ROC analysis. Sem Nucl Med 1978; 8:283–298.
19. Beck JR, Colice GL. Relative operating characteristic analysis applied to tests of pulmonary function. Sem Resp Med 1989; 10:211–217.
20. Beck JB, Schultz EK. The use of relative operator characteristic (ROC) curves in test performance evaluation. Arch Pathol Lab Med 1986; 110:13–20 [Erratum, Arch Pathol Lab Med 1986; 110:958].

Submitted for publication September 1991.
Final revision received November 1991.

Preliminary Report, Case Reports, and Student Corner

Preliminary Report

HIV-1 Seroprevalence in Pregnant Women Testing Positive on Serologic Screening for Syphilis

Rhoda S. Sperling, M.D., Michael Joyner, B.S., Joseph Hassett, M.D., Romina Kee, M.D., Patsy Wheatley, and Carlos Bauer, B.S.

Abstract

Epidemic increases in active syphilis have been reported in the geographic areas hit hard by acquired immunodeficiency syndrome. Although both epidemics have been associated with illicit substance abuse, the extent to which recent increases in syphilis are linked to the human immunodeficiency virus (HIV-1) epidemic is uncertain. In order to define the frequency of syphilis and HIV-1 coinfection in the pregnant patients seen at City Hospital Center at Elmhurst, we saved syphilis-positive serologic specimens from obstetrical patients for anonymous HIV-1 antibody testing. Of 120 women who tested positive for syphilis, 7/120 (5.8%) had antibodies to HIV-1; of the 44 women with VDRL titers ⩾1:16 (suggestive of a recent infection), 1/44 (2.3%) had antibodies to HIV-1.

The effects of coinfections with syphilis and human immunodeficiency virus (HIV-1) in pregnancy and the subsequent risks and manifestations of congenital syphilis are unknown. Given these concerns, it was important to define the frequency of coinfection in our obstetric population; therefore, syphilis-positive serologic specimens from pregnant patients were saved for later anonymous testing for antibodies to the HIV-1 virus.

Materials and Method. From Nov. 1, 1988, through Oct. 31, 1989, all syphilis-positive serologic specimens (positive VDRL and confirmatory FTA) obtained from obstetrical clinic patients, antepartum admissions, and patients in labor were saved for testing for antibodies to the HIV-1 virus. When multiple samples were available for a patient, the most recent sample was used. The highest VDRL titer was recorded for each patient. After it was assured that only a single specimen was obtained per patient, all identifiers were removed from the serum and specimens were anonymously tested by a protocol approved by the Institutional Review Board. Antibody testing was performed on all specimens by standard enzyme-linked immunosorbent assay (HTLV-III ELISA; Electronucleonics, Inc., Columbia, MD). All initially reactive specimens were confirmed by repeat ELISA and Western blot analysis. Western blots were considered positive when there were two or more bands of p24, gp41, or gp 120/160.

Statistical analysis was done by a Fisher's exact test; $p < 0.05$ was considered statistically significant.

Results. From Nov. 1, 1988, through Oct. 31, 1989, 134 obstetrical patients had a positive serologic test for syphilis either antepartum or intrapartum. Of the 134, 120 (90%) had a specimen saved for screening for antibodies to the HIV-1 virus; 7/120 (5.8%) were seropositive for both syphilis and HIV-1 antibodies (95% confidence interval 1.6%–11%.)

A total of 44/120 (37%) had a VDRL titer sometime during gestation that was ⩾1:16, suggesting a recent infection; of these, only one (2.3%) had antibodies to HIV-1 (95% confidence

---

From the Departments of Obstetrics/Gynecology (RS, RK) and Microbiology (PW, CB), City Hospital Center at Elmhurst—Mount Sinai School of Medicine (CUNY) and the Department of Internal Medicine, Division of Clinical Immunology (MJ, JH), Mount Sinai School of Medicine (CUNY), New York, New York. Address reprint requests to Rhoda S. Sperling, M.D., Box 1173, The Mount Sinai Medical Center, One Gustave Levy Place, New York, NY 10029.

interval 0%–6.8%) 76/120 (63%) had a VDRL titer <1:16; of these, six (7.9%) had antibodies to HIV-1 (95% confidence interval 1.8%–14%). The difference in the rates of HIV-1 seroprevalence in the high and low VDRL titer groups was not significant ($p = 0.2$).

Discussion. We do not know the HIV-1 serostatus of the comparable syphilis seronegative patient population during the study period. Based on site-specific data from the ongoing New York State newborn heelstick serosurvey (1), 1.23% of newborns in 1989 had antibodies to HIV-1. Therefore, we estimated that 40–45 HIV-1–infected women delivered liveborn children at our institution during the study period. It appears that the vast majority of HIV-1–infected obstetrical patients at our institution are without serologic evidence of past or present syphilis.

Epidemiologic studies in both Africa and the United States have demonstrated a strong association between evidence of past syphilis and the risk of HIV-1 infection (2–6). Syphilis and other genital ulcer diseases result in disruption of epithelial or mucosal surfaces which may provide a portal of entry for the virus into the systemic circulation. In our pregnant population, 5.8% of women with positive syphilis serology also had antibodies to HIV-1. As we lack risk behavior data for both the syphilis seropositive and seronegative population, we cannot ascertain whether a positive syphilis serology is an independent risk factor for HIV-1 acquisition in our population. A much larger patient cohort followed over time will be required to more accurately answer this important question.

References

1. Novick LF, Berns D, Stricof R, et al. HIV seroprevalence in newborns in New York State. JAMA 1989; 261:1745–1750.
2. Simonsen JN, Cameron W, Gakinya MN, et al. Human immunodeficiency virus infection among men with sexually transmitted diseases experience from a center in Africa. N Engl J Med 1988; 319:274–278.
3. Quinn TC, Glasser D, Cannon RO, et al. Human immunodeficiency virus infection among patients attending clinics for sexually transmitted diseases. N Engl J Med 1988; 318:197–203.
4. Greenblatt RM, Lukehart SA, Plummer FA, et al. Genital ulceration as a risk factor for human immunodeficiency virus infection. AIDS 1988; 2:47–50.
5. Darrow WW, Echenberg DF, Jaffe HW, et al. Risk factors for human immunodeficiency virus (HIV) infection in homosexual men. Am J Public Health 1987; 77:479–483.
6. Stamm WE, Handsfield HH, Rompalo AM, et al. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. JAMA 1988; 260:1429–1433.

Submitted for publication August 1990.
Revision received November 1990.

Phycomycosis (Mucormycosis) in Differential Diagnosis of Cerebral Mass Lesions in Intravenous Drug Users

Arthur A. Gaing, M.D., Francisco Corbalan, M.D., and Jesse Weinberger, M.D.

To our knowledge, only seven cases of focal cerebral mucormycosis in association with intravenous drug use have been reported in the English-language literature (1–6). We describe a case in a nonimmunocompromised host whose survival was prolonged.

Case Report

A 53-year-old woman, an active intravenous cocaine user, was admitted to the hospital for progressive disorientation, difficulty in speech, and right-sided weakness. Her medical history was noncontributory. Her only medication was methadone, 40 mg/day, for prior heroin addiction.

On examination, her temperature was 38°C (100.2°F); other vital signs were normal. The patient had neck stiffness, expressive aphasia, right facial palsy, and right hemiparesis. Examination of the heart, lungs, and abdomen was unremarkable. Complete blood count, SMA 12, urinalysis, chest X-ray, and two-dimensional echocardiography were all negative. A computed tomography scan of the head with intravenous contrast (Fig. 1) showed multiple enhancing lesions in the right frontal lobe, left basal ganglia, and left internal capsule with a mass effect on the third ventricle and obstructive hydrocephalus of the lateral ventricles proximally. At lumbar puncture, opening pressure was 80 mm; cerebrospinal fluid protein 76 mg/dL; glucose 74 mg/dL with red blood cells 100/mm^3 and white blood cells 600/mm^3 (26% polymorphonuclear and 74% mononuclear cells). India ink preparation, Gram stain, and stain for acid-fast bacilli were negative. Subsequent cultures were all negative. Antibodies to human immunodeficiency virus were not present. T4 lymphocytes were 903/mm^3 (43%), T8 lymphocytes 693/mm^3 (33%), T4/T8 ratio 1.3 (normal 0.9–2.94).

Anti-*Toxoplasma* medications (sulfadiazine, 1 g every 6 hr, and pyrimethamine, 25 mg/day) and intravenous dexamethasone were started empirically. Seizures were controlled with dilantin. Repeat tomographic scan of the head one month later showed no improvement. Rifampin, isoniazide, pyrazinamide, and pyridoxine were added. The patient remained hospitalized, and three months after admission a right frontal lobe biopsy showed nonseptated hyphae consistent with an agent of phycomycosis (Fig. 2). Marked tissue necrosis, hemorrhage, acute and chronic inflammatory exudate, and gliosis were present. There was crowding of fungal hyphae around the blood vessels but thrombi containing fungus were not seen. Culture of the biopsy failed to grow the organism. Intravenous amphotericin B therapy was begun; when 2 g had been administered, computed tomography scan of the head revealed marked reduction in the size of the lesions. The patient's mental status and hemiparesis also improved. She was discharged to a chronic care facility and is alive and well seven months after completion of amphotericin B therapy.

Discussion

Phycomycosis is an opportunistic infection caused by nonseptate fungi belonging to the class

---

From the Department of Internal Medicine, North General Hospital, New York, NY. FC's current address is Department of Geriatrics, The Mount Sinai Hospital, One Gustave L. Levy Place, New York, NY 10029. Address reprint requests to Arthur A. Gaing, M.D., Department of Gastroenterology, Box 1069, The Mount Sinai Hospital, One Gustave L. Levy Place, New York, NY 10029.

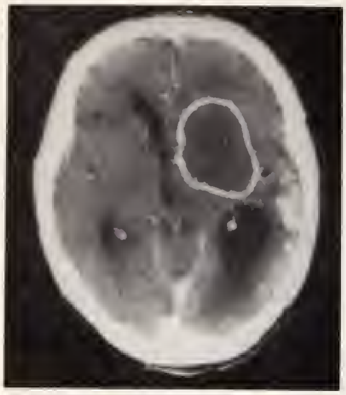
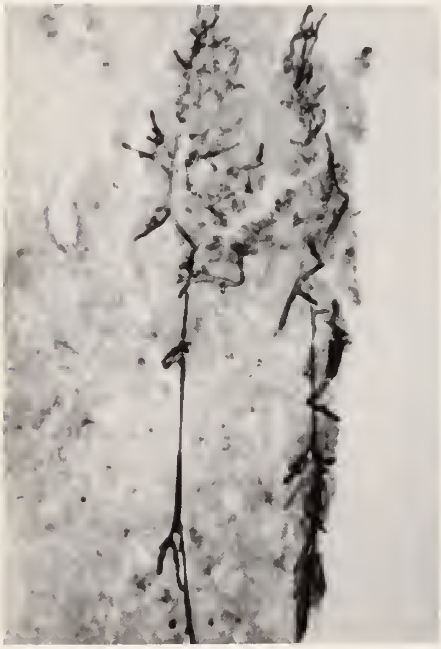


Fig. 1. Above Computed tomographic scan of brain with contrast material showing large lesion involving left basal ganglia and left internal capsule with dilatation of ventricular system.

Fig. 2. Below Touch preparation of brain biopsy showing nonseptate, right-angle branching hyphae consistent with a Phycomycete (Gomori silver methanamine stain ×400).



Zygomycetes (Phycomycetes) and the order Mucorales. The Zygomycetes are ubiquitous and generally saprophytic, rarely causing disease in otherwise healthy persons. The five major forms of mucormycosis are rhinocerebral, pulmonary, disseminated, gastrointestinal, and cutaneous, each associated with specific abnormalities in host defense mechanisms (7). Acidosis and diabetes mellitus have been associated with 40% of all cases and 70% of cases with rhinocerebral involvement. Pulmonary and disseminated forms occur in association with leukopenia, immunologic suppression, and burns. The gastrointestinal form occurs in noncompromised patients and in patients with debilitating diseases.

The mortality rate in patients with central nervous system infection is more than 85%. The organism invades the nasal sinuses or palate and extends into the paranasal sinuses. The infection progresses through the ethmoids and spreads to the retroorbital region. It reaches the brain by extending directly through the cribriform plate or paranasal sinuses into the meninges or by spreading along the blood vessels and lymphatics. Sometimes there is no evidence of portal of entry.

To our knowledge only 19 cases of focal cerebral mucormycosis have been reported (1, 2, 6). Seven cases in association with intravenous drug use have been described (1–6) (including one patient with AIDS); 1 with AIDS who was not an intravenous drug user; 3 with open head trauma and or surgery; 3 with renal insufficiency; 1 with corticosteroid therapy; and 4 with no underlying risk factors. Phycomycosis causing obstructive hydrocephalus has been reported only twice (1, 8), and one patient who was not a drug addict had a chronic course and survived. Phycomycetes have a marked predilection for invasion of the walls of large and small arteries, leading to thrombosis with extension of infection, tissue necrosis, and distal embolization. In the cases associated with intravenous drug use, the fungus is probably injected intravenously or enters the bloodstream from an infected injection site. Transient pulmonary infection occurs, with subsequent embolization to the brain. Acidosis, usually caused by diabetes, promotes the growth of this fungus. After obstruction of the arteries, there may be an immediate local acidosis from focal ischemia which provides optimal conditions for a fulminant course of the infection.

Fever, headache, nuchal rigidity, nausea, vomiting, confusion, and focal neurologic deficits are features of cerebral phycomycosis. The cerebrospinal fluid, computed tomography scan, and electroencephalogram are often abnormal, but not diagnostic. Brain biopsy and culture is generally required for diagnosis; although Marchevsky et al. reported positive cultures in 13 of their 14 cases diagnosed during life, all of their specimens were from extracerebral sites (9). Many authors found poor yield from cerebral tissue cultures obtained during life or at the time of autopsy (1, 8, 10). Therapy consists of systemic administration of amphotericin B and, if possible, excision of as much infected tissue as possible.

Differential diagnosis of cerebral mass lesions in an intravenous drug user includes toxoplasmosis, tuberculosis, cryptococcoma, aspergilloma, herpes simplex infection, Kaposi's sarcoma, lymphoma, and progressive multifocal leukoencephalopathy. Phycomycosis should be included in this differential diagnosis whether or not the patient is immunocompromised. Because noninvasive tests are nonspecific, an early brain biopsy is essential for initiation of definitive therapy to improve outcome.

References

1. Woods KF, Hanna BJ. Brain stem mucormycosis in a narcotic addict with eventual recovery. Medicine 1986; 80:126–128.
2. Cuadrado LM, Guerrero A, Lopez Garcia Asenjo JA, Martin F, Palau E, Garcia Urra D. Cerebral mucormycosis in two cases of acquired immunodeficiency syndrome. Arch Neurol 1988; 45:109–111.
3. Adelman LS, Aronson SM. The neuropathologic complications of narcotics addiction. Bull NY Acad Med 1969; 45:225–234.
4. Hameroff SB, Eckholdt JW, Lindenberg R. Cerbral phycomycosis in a heroin addict. Neurology 1970; 20:261–265.
5. Masucci EF, Fabara JA, Saini M, Kurtzke JF. Cerebral mucormycosis (phycomycosis) in a heroin addict. Arch Neurol 1982; 39:304–306.
6. Pierce PF Jr, Solomon SL, Kaufman L, et al. Zygomycetes brain abscesses in narcotic addicts with serological diagnosis. JAMA 1982; 248:2881–2882.
7. Parfrey NA. Improved diagnosis and prognosis of mucormycosis, a clinicopathologic study of 33 cases. Medicine 1986; 65:113–123.
8. Sweeney PJ, Hahn JF, McHenry MC, Mitsumoto H. Mucormycosis presenting as positional nystagmus and hydrocephalus. J Neurosurg 1980; 52:270–272.
9. Marchevsky AM, Bottone EJ, Geller SA, et al. The changing spectrum of disease, etiology and diagnosis of mucormycosis. Human Pathology 1980; 11:457–464.
10. Meyer RD, Rosen P, Armstrong D. Phycomycosis complicating leukemia and lymphoma. Ann Intern Med 1972; 77:871–879.

Submitted for publication August 1990.
Revision received November 1990.

Cortical Blindness in Postpartum Preeclampsia Progressing to Eclampsia:

Case Report

David Mandelkern, M.D., and Andrew Burger, M.D.

Abstract

A 23-year-old woman who had an uneventful prenatal course and normal delivery developed severe, generalized headache and blurred vision on postpartum day four. The patient was noted to have generalized hyperreflexia and sustained ankle clonus. The blood pressure was 170/100 mm Hg, there was no edema, and the urine showed trace proteinuria. The visual disturbance rapidly progressed to complete blindness with preserved pupillary reactions. The patient then had a generalized tonic-clonic seizure lasting about one minute. Treatment was initiated with intravenous diazepam and phenytoin, and there was no recurrence of seizure activity. Vision returned to normal and the patient made a complete recovery. This case is presented to demonstrate progressive postpartum preeclampsia and the importance of early recognition and treatment. Pathophysiologic mechanisms and treatment options are discussed.

Preeclampsia is a disorder that occurs in pregnancy and the puerperium. It has been defined as "a hypertensive disorder accompanied by proteinuria, edema, and, occasionally, coagulation abnormalities" (1). Eclampsia is the development of seizures or coma following preeclampsia. Cerebral vasospasm with alteration in blood-brain barrier permeability may be involved in the pathophysiology of eclampsia. Preeclampsia must be considered in the differential diagnosis of postpartum patients with headache and visual disturbance. Physicians should be aware of the features of postpartum preeclampsia, given the possibility of adverse consequences if not recognized. A case of cortical blindness in postpartum preeclampsia progressing to eclampsia is presented. If treatment had been initiated earlier, the progression of symptoms might have been halted.

Case Report

A 23-year-old woman, gravida 1 para 0, was admitted in labor at 39 weeks' gestation. The patient had a history of migraine headaches, but no personal or family history of seizures. The prenatal course was uneventful. On admission, the blood pressure was 120/70 mm Hg, there was no edema, and the urine dipstick showed trace proteinuria. The patient underwent epidural anesthesia and low-forceps delivery. Apgar test score was 9/10. On postpartum day two, the patient reported an occipital headache, which was thought to be secondary to a wet tap from the epidural anesthesia, and she was treated with oxycodone and intravenous hydration. The blood pressure was 140/90 mm Hg. On postpartum day three, the blood pressure was 170/80 mm Hg, and a toxemia screen was initiated. The reflexes were noted to be normal, there was no edema, and trace proteinuria was present. The patient reported an episode of seeing spots. On postpartum day four, the patient reported severe, generalized headache and blurred vision.

A neurologic consultation was obtained. The

From the Department of Neurology, Mount Sinai School of Medicine (CUNY), New York, NY. Address correspondence and reprint requests to David Mandelkern, M.D., 1249 Park Avenue #4A, New York, NY 10029.

blood pressure was 170/100 mm Hg. The neck was supple. The patient was alert and fully oriented. She was unable to read with the left eye, but the visual acuity was 20/200 on the right. Visual fields were full, and funduscopic examination revealed normal discs. Pupils were equal and reactive to light, and eye movements were intact. The remaining cranial nerves were normal, as were the motor and sensory examination results. Generalized hyperreflexia was present, with sustained ankle clonus. Babinski sign was absent.

The visual disturbance rapidly progressed to complete blindness with preserved pupillary reactions. The head deviated to the left, followed by right facial twitching lasting several seconds. The patient then had a generalized tonic-clonic seizure lasting about one minute. The patient received 10 mg intravenous diazepam followed by 1 g intravenous phenytoin administered over an hour.

After the seizure, optokinetic nystagmus was absent. The vision gradually returned, although it was blurred. Within several hours the patient could distinguish colors and count fingers. She was still hyperreflexic, but the clonus had resolved. The blood pressure decreased to 116/78 mm Hg. There was no recurrence of seizures. The patient was treated with maintenance phenytoin, 300 mg taken orally each day, with a phenytoin level of 23 mg/L. On postpartum day five, the visual acuity became 20/20 in both eyes.

On lumbar puncture after the seizure, opening pressure was 12 cm water; glucose was 54 mg/dL, protein 60 mg/dL, leukocyte count $1/mm^3$, red cell count $4/mm^3$. A computed tomogram of the head, with and without intravenous contrast, performed on the day of seizure was normal. Results of electroencephalogram and visual evoked potentials done on postpartum day six were normal.

Other laboratory data included serum creatinine 0.8 mg/dL, uric acid 5.3 mg/dL, and platelet count 280,000. Hepatic enzymes (in IU/L) were as follows: serum glutamic oxaloacetic transaminase 16, serum glutamic pyruvic transaminase 14, alkaline phosphatase 157.

The patient had no further complications and was discharged on postpartum day seven.

Discussion

Pathophysiologic Mechanisms. The pathophysiology of eclampsia is incompletely understood. Cunningham et al. theorize that hypertension in preeclampsia is caused by increased resistance to blood flow as a result of increased constriction at the microvascular level (2). Although it had previously been found that vascular resistance increases and cardiac output decreases in preeclampsia and eclampsia, a subset of patients have normal vascular resistance and increased cardiac output. Eclamptic patients develop increased vascular sensitivity to angiotensin II, leading to vasospasm. Evidence of vasospasm has been demonstrated by cerebral angiography showing spasm of large- and medium-caliber arteries in a patient with postpartum eclampsia whose preeclamptic state progressed to stupor with focal neurologic findings (3). However, because the angiogram was performed after eclampsia developed, it is possible that the vasospasm may not have been involved in the pathogenesis of the eclampsia.

Incidence in Pregnancy, During Delivery, and Postpartum. Preeclampsia occurs in 5% of pregnancies, and the incidence of eclampsia is 1 in 1,150 deliveries (2). Eclampsia itself is associated with a risk of mortality. It is therefore important to identify the clinical findings that portend a fulminant progression to eclampsia.

The potential for the development of eclampsia in the postpartum state may be overlooked, as it was in our patient. In a series of 132 eclamptic patients observed by Watson et al., postpartum onset occurred in 36 (27%) (4). Of the postpartum eclamptics, 17 (47%) had onset more than 48 h after delivery. In a series reported by Sibai et al. of 65 patients who developed eclampsia, onset was postpartum in 14 (21%) (5).

Clinical Signs. In the case reported here, headache, visual disturbance, hyperreflexia, and ankle clonus in a patient with hypertension and trace proteinuria were clear indications of preeclampsia. The diagnoses of migraine headache or transient cerebral ischemia can be excluded, given this clinical picture of hyperreflexia with ankle clonus and progression to sustained generalized seizure. The patient had no evidence of cerebral hemorrhage or venous occlusion, as evidenced by normal findings on computed tomography of the head and lumbar puncture.

Visual disturbance and headache are common complaints in preeclampsia prior to the development of eclampsia. In Watson's series of late postpartum eclampsia, all 17 patients had visual disturbance and headache prior to seizures. Sibai reports transient cortical blindness occurring in three patients of his series (5%). Grimes et al. (6) report a patient who developed sudden headache and total blindness at 41 weeks' gestation, with a computed tomogram of the head showing low-density areas in the occipital region. An electroencephalogram was abnormal, showing posterior slowing and epileptiform activity. After delivery of the fetus, the patient's vision improved. Leibowitz and Hall (7) report on another patient who developed headache and blindness at 40 weeks' gestation; computed tomogram of the head showed multiple small, hypodense lesions in the basal ganglia and occipital lobe. Again, after delivery, vision returned to normal. Both these patients were treated early with magnesium sulfate and did not progress to eclampsia. They also present compelling evidence that the visual disturbance resulted from occipital lobe dysfunction.

The patient reported on here had normal findings on computed tomogram of the head, electroencephalogram, and visual evoked potential. In Sibai's series, the scans were normal, but 75% of the electroencephalograms showed either diffuse or focal slow activity with 10% paroxysmal spike activity; EEG findings all became normal within six months. Brown et al. (8) found a 29% (14/49) rate of abnormal findings in computed tomograms of the head, often showing areas of hypodensity in the occipital region, and pointed out that the scans in Sibai's study were done before higher-resolution equipment was available.

Treatment Options. In the case reported here, intravenous phenytoin was given when the patient had a seizure. The use of a phenytoin in eclampsia has been previously reported. In a series by Slater et al. (9), 26 patients with preeclampsia and eclampsia were treated with intravenous phenytoin. None had a convulsion following the treatment.

Magnesium sulfate has been the drug of choice for treatment of eclampsia because of its long-standing use and general success. However, treatment failures have been reported with magnesium sulfate. In the series by Watson et al. (4), two patients with postpartum preeclampsia developed seizures while receiving intravenous magnesium sulfate with levels in the therapeutic range. In the series by Sibai et al. (5), one patient developed intrapartum eclampsia while receiving magnesium sulfate. Moreover, the usefulness of magnesium sulfate has never been demonstrated in a controlled clinical trial, and it has been argued convincingly that there are better methods of treating the seizures associated with eclampsia (10, 11).

Intravenous diazepam followed by the loading of intravenous phenytoin should be used to treat seizures in eclampsia.

An antihypertensive agent should be used early in an attempt to prevent the cascading events that lead to eclampsia.

Hydralazine has been effective in the treatment of preeclampsia (2, 12). It should be started when the blood pressure rises significantly above the patient's baseline value.

Prenatal care should be emphasized for early detection of this condition.

In general, eclampsia is preventable and is almost always preceded by preeclampsia (2). However, as in the patient reported on here, symptoms of preeclampsia may be mild, although ominous neurologic symptoms of visual disturbance and headache, as well as hyperreflexia, may be present. Sustained ankle clonus is a sign of impending eclampsia (13).

Our recommendation is that when a patient with preeclampsia develops visual disturbance, headache, and hyperreflexia, prevention of seizures should begin by treating with a loading dose of phenytoin. Phenytoin levels should be followed and maintained in the therapeutic range while the patient is preeclamptic. Early commencement of treatment at the onset of these findings is essential if progression to eclampsia is to be minimized or avoided.

References

1. Dinsdale HB. Does magnesium sulfate treat eclamptic seizures? Yes. Arch Neurol 1988; 45:1360–1361.
2. Cunningham FG, MacDonald PC, Gant NF. Hypertensive disorders in pregnancy. In: Williams obstetrics. 18th ed. Norwalk, CT: Appleton & Lange, 1989:653–694.
3. Trommer BL, Homer D, Mikhael MA. Cerebral vasospasm and eclampsia. Stroke 1988; 19:326–329.
4. Watson DL, Sibai BM, Shaver DC, et al. Late postpartum eclampsia: an update. South Med J 1983; 76:1487–1489.
5. Sibai BM, Spinnato JA, Watson DL, et al. Eclampsia. IV. Neurological findings and future outcome. Am J Obstet Gynecol 1985;152:184–192.
6. Grimes DA, Ekbladh LE, McCartney WH. Cortical blindness in preeclampsia. Int J Gynaecol Obstet 1980; 17:601–603.
7. Liebowitz HA, Hall PE. Cortical blindness as a complication of eclampsia. Ann Emerg Med 1984; 13:365–367.
8. Brown CEL, Purdy P, Cunningham FG. Head computed tomographic scans in women with eclampsia. Am J Obstet Gynecol 1988; 159:915–920.
9. Slater RM, Smith WD, Patrick J, et al. Phenytoin infusion in severe pre-eclampsia. Lancet 1987; 1:1417–1421.
10. Kaplan PW, Lesser RP, Fisher RS, et al. No, magnesium sulfate should not be used in treating eclamptic seizures. Arch Neurol 1988; 45:1361–1364.
11. Kaplan PW, Lesser RP, Fisher RS, et al. A continuing controversy: magnesium sulfate in the treatment of eclamptic seizures. Arch Neurol 1990; 47:1031–1032.
12. Olanow CW. Neurologic aspects of pregnancy. In: Merritt's textbook of neurology. 8th ed. Philadelphia, London: Lea & Febiger, 1989:892–897.
13. Turnbull A, Chamberlain G. Hypertension in pregnancy. In: Obstetrics. Edinburgh, New York: Churchill Livingstone, 1989:515–541.

Submitted for publication February 1991.

Duodenocolic Fistula: An Unusual Presentation of Esophageal Squamous Cell Carcinoma

PETACHIA REISSMAN, M.D., RANDOLPH M. STEINHAGEN, M.D., AND PATRICK F. ENRIGHT, M.D.

Abstract

Duodenocolic fistula, one of the less common forms of entero-enteric fistulas, is usually associated with colonic malignancies at the hepatic flexure or Crohn's disease of the colon or duodenum. We report an unusual case of duodenocolic fistula caused by metastatic esophageal squamous cell carcinoma in which the primary esophageal tumor remained asymptomatic. In several collected series of clinical and autopsy studies of over 2800 patients, no similar complication of metastatic esophageal squamous cell carcinoma has been reported.

DUODENOCOLIC fistula (DCF) is one of the less common forms of entero-enteric fistula. The most frequent causes are primary malignancies of the colon (1–4) and Crohn's disease involving the colon or duodenum (5, 6). Less common causes include perforated duodenal ulcer (7), perforated duodenal diverticulum (8–10), ruptured appendicitis (11), acute cholecystitis (12), tuberculous lymphadenitis (13), carcinoma of the gallbladder eroding into the duodenum and colon (3), and inadvertent injury to the duodenum and colon during gastric surgery (14).

We present a case of DCF caused by a metastatic deposit from a squamous cell carcinoma of the esophagus. This fistula was responsible for all of the patient's symptoms.

Case Report. A previously healthy 53-year-old black man was evaluated for a two-month history of cramping abdominal pain, diarrhea, and a 30-pound weight loss. On physical examination the patient was cachectic and febrile to 38.2°C. The abdomen was soft, but there was a tender 10-cm mass in the right upper quadrant. The stool was guaiac negative. The white-cell count was 18,000 with 88% neutrophils; the hemoglobin was 10.3 gm/dL. The alkaline phosphatase was 170 mg/dL, the GGTP 185 mg/dL, and the albumin 3.3 g/dL.

Barium enema examination (Fig. 1) demonstrated a fistula between the right transverse colon and the second portion of the duodenum. There was retrograde filling of the proximal duodenum and stomach. The colonic mucosa at the site of the fistula appeared to be normal. An upper-gastrointestinal swallow showed a longitudinal nonobstructing, irregular tumor in the mid-esophagus (Fig. 2). There were inflammatory changes of the duodenal mucosa, and a DCF was seen communicating between the second portion of the duodenum and the transverse colon (Fig. 3). Upper endoscopy confirmed the presence of a nonobstructing mid-esophageal tumor; biopsies revealed poorly differentiated squamous cell carcinoma. Colonoscopy identified the fistula site in the transverse colon, but did not find any intraluminal pathology. CT scan of the chest demonstrated the esophageal tumor to be arising from the mid-esophagus. No involvement of adjacent mediastinal structures was seen. Abdominal CT scan (Fig. 4) showed a large abscess in the area of the porta hepatis, with evidence of para-aortic

---

From the Departments of Surgery (PR, RMS) and Radiology (PFE), The Mount Sinai Medical Center, New York, NY, and The Veterans Affairs Medical Center, Bronx, New York. Address reprint requests to Randolph M. Steinhagen, M.D., Department of Surgery, Box 1259, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

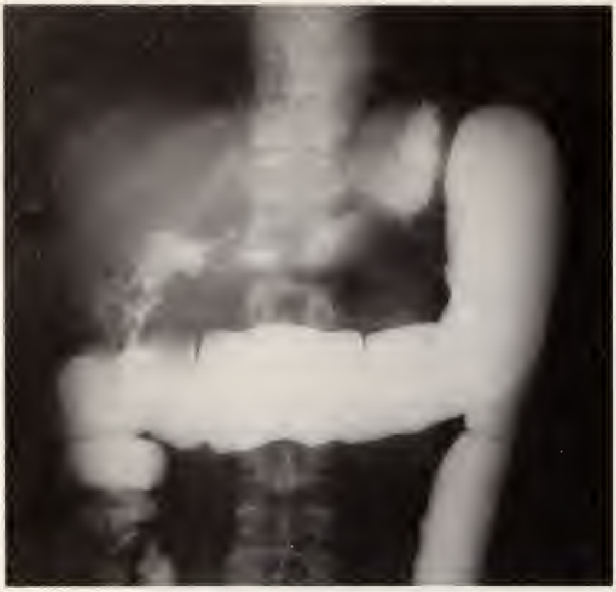


Fig. 1. Barium enema study demonstrating fistula between right transverse colon and second portion of duodenum. Colonic mucosa at fistula site appears normal. Note retrograde filling of duodenum and stomach.

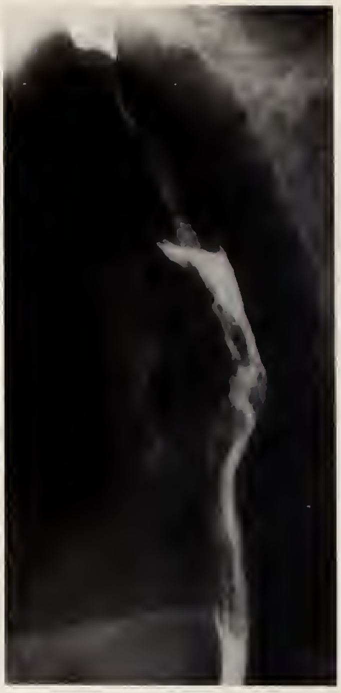


Fig. 2. Esophagram demonstrating longitudinal, irregular lesion in mid-esophagus. Note absence of proximal dilatation, indicating that lesion is not obstructing.

lymphadenopathy. No liver metastases were seen.

The patient remained febrile, with right upper quadrant tenderness and persistent leukocytosis. At laparotomy, a large abscess consisting of necrotic tumor, pus, and fecal material was found under the right lobe of the liver. A portion of the antimesenteric wall of the right transverse colon and most of the anterior wall of the second portion of the duodenum were replaced by necrotic tumor, and were in communication via the abscess cavity. Enlarged para-aortic lymph nodes were palpable. After evacuation of the abscess and a large portion of the necrotic tumor, a right hemicolectomy was performed. In the face of the large defect in the anterior duodenal wall, the stomach was divided at the level of the pylorus, and a gastrojejunostomy was constructed. A tube duodenostomy was inserted, and drains were placed in the right paracolic gutter.

Pathological examination of the resected

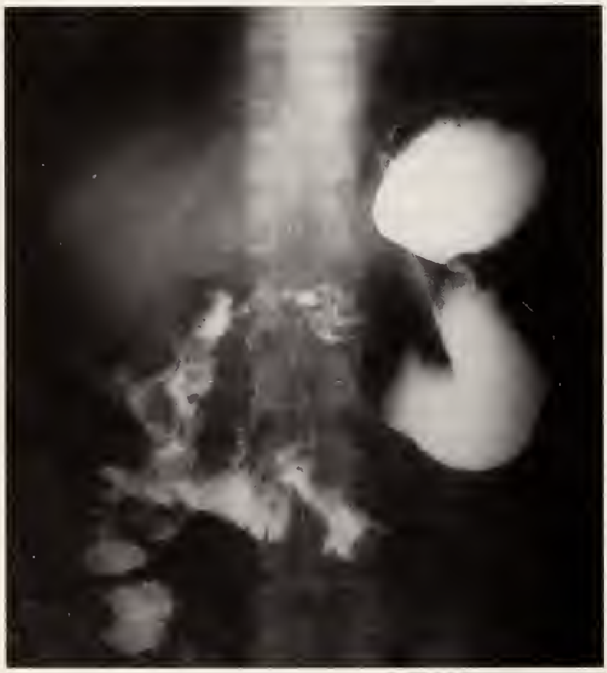


Fig. 3. Upper gastrointestinal study demonstrating normal-appearing stomach, hypertrophic duodenal mucosa, indicating chronic inflammatory reaction, and passage of barium into transverse colon via fistula.

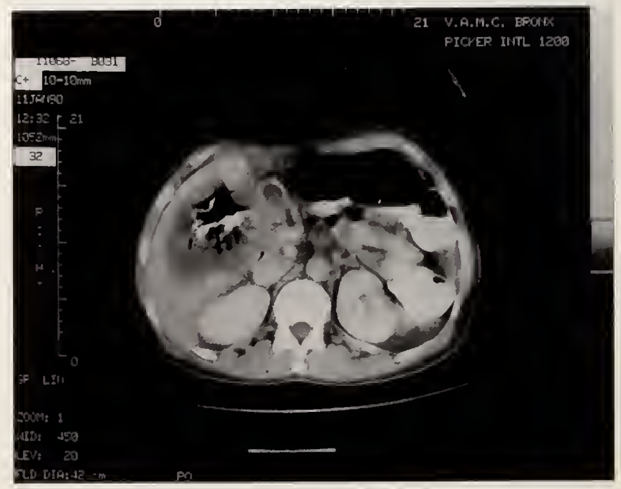


Fig. 4. Abdominal CT scan with oral and intravenous contrast demonstrating large fluid collection containing air and extravasated contrast. This abscess is located in region of porta hepatis.

specimen revealed metastatic, poorly differentiated squamous carcinoma.

The early postoperative course was uneventful. The duodenostomy output gradually decreased and the patient was able to eat a regular diet. Approximately eight weeks after surgery the patient first began to experience dysphagia, and radiotherapy to the primary tumor in the esophagus was initiated. The patient died approximately three months after surgery.

Discussion. Because of anatomical proximity, most duodenocolic fistulas occur between the second or third portions of the duodenum and the hepatic flexure of the colon (1–3). However, the first portion of the duodenum and the ascending colon or cecum may be involved as well (5, 11). The most common cause is carcinoma of the hepatic flexure infiltrating into the duodenum (1–3). The estimated incidence due to transverse colon carcinoma is one in 900 cases (1).

DCF is also seen in association with Crohn's disease, although the duodenum is one of the less frequent fistula sites in this disease (5, 6). Other causes of DCF are uncommon and have been reported only as isolated cases. There has not, however, been a previously reported case of DCF caused by metastatic carcinoma from a primary lesion in the esophagus. Although esophageal tumors have been known to spread to other portions of the gastrointestinal tract below the diaphragm via direct extension (15–18), in a clinical study of 478 patients with esophageal squamous cell carcinoma, none had distant gastrointestinal metastases. In several collected series of autopsy studies of 2,850 patients with carcinoma of the esophagus, distant metastases occurred most frequently in thoracic and infradiaphragmatic lymph nodes (73–77%); lungs (31–52%); and liver (21–47%). Other less common abdominal metastases included peritoneum (3.5%), kidneys (5.1%), adrenals (3.2%), gastric wall (2.6%), spleen (1.4%), pancreas (1.2%), and small and large intestine (0.9%) (15, 17, 18). However, none of the gastrointestinal metastases were associated with any form of enteric fistula formation. In the case presented here, the DCF was caused by a metastatic lesion and not by direct extension of the primary tumor.

Although the clinical manifestations of DCF may vary according to the primary underlying disorder, several common clinical features, resulting from the abnormal passage of colonic contents into the duodenum, have been described. These symptoms include cramping abdominal pains, malabsorption, and weight loss (1, 2, 5, 8, 10), and are thought to be caused by the presence of colonic organisms within the upper small intestine. Less frequently, gastrointestinal bleeding, feculent vomiting, or the presence of a palpable mass caused by a perifistula abscess have been described (1–3, 9, 11).

Barium enema examination is usually the most reliable test for demonstrating the presence of DCF, and it will frequently demonstrate associated pathology in the colon as well (1–3, 5, 11). The direction of flow through the fistula is usually from colon to duodenum because of higher pressures within the colon, therefore upper gastrointestinal contrast studies are far less accurate (2, 11). Endoscopic examination of the stomach, duodenum, and colon should be performed in the search for the underlying etiology once DCF has been identified.

Most authors agree that surgical correction should be undertaken as soon as the patient can tolerate the procedure (1–3, 5–6). In many cases, intravenous hyperalimentation, correction of anemia, and correction of electrolyte imbalance may be required preoperatively (1, 2, 5, 8).

The surgical approach to DCF varies according to the primary cause. For benign conditions, the majority of authors suggest simple closure of the duodenal defect and right hemicolectomy with ileotransverse anastomosis (3, 5, 6, 8, 9, 11). Other procedures suggested for benign DCF include primary repair of both the duodenum and colon with proximal diversion (6, 7), simple repair of the duodenum with resection of the involved colon (12), and use of a jejunal serosal patch or duodenojejunostomy when the duodenal defect is too large to close primarily (1, 2, 5). It has also been suggested that repair should be accomplished in stages, the initial procedure consisting only of fecal diversion, and subsequent resection being undertaken only after the inflammatory reaction was subsided (19).

When DCF is associated with primary colon carcinoma, several surgical procedures have been suggested. Right hemicolectomy is obviously always required. This can be combined with resection of the anterior duodenal wall and simple closure of the defect if it is small enough, or duodenojejunostomy or jejunal patch for larger defects (2, 3). Alternatively, the duodenum may be closed and intestinal continuity reestablished via gastrojejunostomy (1), or the most radical option, combining right hemicolectomy with pancreatoduodenectomy, may be considered (1, 2). In the presence of metastases or locally advanced tumor, a palliative bypass procedure consisting of gastrojejunostomy and side-to-side ileotransverse anastomosis can be performed (1, 3).

In this case, the DCF and its associated abscess was the main source of the patient's symptoms. As is often the case, the procedure was dictated by the local findings. Controlled duodenal fistula via tube duodenostomy, with pyloric division and gastrojejunostomy were felt to be the safest alternatives. Obviously, a right hemicolectomy was also required. The output from the duodenostomy was easily managed and became negligible over several weeks. The patient was able to eat until the time of his death.

In this case, DCF was caused by, and in fact was the initial symptom of, metastatic squamous carcinoma of the esophagus. This is unique in two respects. The first is the fact that the fistula was caused by a metastatic lesion rather than a primary colonic neoplasm. The second is the finding of intestinal metastases from a squamous carcinoma of the esophagus.

References

1. Welch JP, Warshaw AL. Malignant duodenocolic fistulas. Am J Surg 1977; 133:658–661.
2. Vieta JO, Blanco R, Valentini GR. Malignant duodenocolic fistula: report of two cases, each with one or more other synchronous gastrointestinal cancers. Dis Colon Rectum 1976; 19:542–552.
3. Calvert DG, Medhurst GA. Fistula formation between the duodenum and colon: a report of three cases and a review of the condition. Br J Surg 1960; 48:136–139.
4. Raffensperger EC, Sylvester LE, Ferguson LK. Duodenocolic fistula due to carcinoma of the hepatic flexure: report of a case with a discussion of the surgical problems encountered. Arch Surg 1957; 74:333–336.
5. Lee KKW, Schraut WH. Diagnosis and treatment of duodenoenteric fistulas complicating Crohn's disease. Arch Surg 1989; 124:712–715.
6. Jacobson IM, Schapiro RH, Warshaw AL. Gastric and duodenal fistulas in Crohn's disease. Gastroenterology 1985; 89:1347–1352.
7. Herrington JL Jr. Duodenocolic fistula with perforation secondary to peptic ulcer. Am J Surg 1967; 113:285–288.
8. Dey KR, Latchmore AJC. Steatorrhoea due to duodenocolic fistulae from perforation of a duodenal diverticulum with ectopic gastric epithelium. Br J Clin Pract 1970; 24:444–445.
9. Sarris SP. Case records of the Massachusetts General Hospital, Case 46091. N Engl J Med 1960; 262:462–466.
10. Hughes S, Marsh MN. Fistulating duodenal diverticulum with associated vitamin B_{12} deficiency. Postgrad Med J 1983; 59:536–538.
11. Clayton RS, Thornton WL. Benign duodenocolic fistula: review of the literature and case report. Radiology 1953; 60:832–836.
12. Neville WE. Duodenocolic fistula due to acute cholecystitis. Am J Surg 1954; 87:300–302.
13. Ogilvie H. Non-malignant duodeno-colic fistula. Ann Surg 1950; 131:899–902.
14. Hux RH, Zuska JJ. Benign duodenocolic fistula: report of a case and review of the literature on fistula as a complication of subtotal gastric resection. Arch Surg 1962; 84:564–569.
15. Postlethwait RW. Squamous cell carcinoma of the esophagus. In: Postlethwait RW, ed. Surgery of the esophagus. Norwalk, CT: Appleton-Century-Crofts, 1986; 369–442.
16. McKeown, KC. Clinical presentation of carcinoma of the oesophagus. J Royal Coll Surg (Edinb) 1986; 31:199–206.
17. Anderson LL, Lad TE. Autopsy findings in squamous-cell carcinoma of the esophagus. Cancer 1982; 50:1587–1590.
18. Mandard AM, Chasle J, Marnay J, Villedieu B, Bianco C, Roussel A. Autopsy findings in 111 cases of esophageal cancer. Cancer 1981; 48:329–335.
19. Leichtling JJ, Garlock JH. Granulomatous colitis complicated by gastrocolic, duodenocolic and colopulmonic fistulas. Gastroenterology 1962; 43:51–165.

Submitted for publication October 1991.
Final revision received November 1991.

Hyperthyroidism in Patients Treated with Lithium:

Report of Nine Cases

David K. Sirota, M.D., Rhoda H. Cobin, M.D., Walter Futterweit, M.D., Robert L. Segal, M.D.,
David R. Jacobs, M.D., and Edward Merker, M.D.

Abstract

Nine cases of hyperthyroidism which developed in patients on lithium therapy are presented and analyzed and the literature is briefly reviewed. The findings strongly suggest that lithium therapy does not cause hyperthyroidism.

SINCE the introduction of long-term lithium therapy for psychiatric disorders, an association between this therapy and a variety of thyroid diseases has been noted. These include nontoxic goiters (1), autoimmune thyroiditis (2), hypothyroidism (3–5), thyroid carcinoma (6), and endocrine exophthalmos (7). Thyrotoxicosis has also been reported to occur in patients on lithium therapy (3, 8–21). As of 1986, only 25 such cases had been reported in the world literature (21, 22). The present report adds 9 new cases to the literature and attempts to further characterize this interesting association.

Materials and Methods. Between 1972 and 1987 the diagnosis of hyperthryoidism was made in 9 patients who were taking lithium to control psychiatric illness. Characteristic physical stigmata of thyrotoxicosis or the ophthalmologic findings of Graves' disease were found in each. Laboratory studies, including elevated thyroid hormone levels, elevated radioactive iodine uptake, impaired TSH response to TRH or elevated TSI confirmed the diagnosis in every case. These cases were analyzed with respect to age, sex, duration of lithium therapy, personal or family history of thyroid disorders or other autoimmune disease, physical findings, and associated laboratory data suggestive of autoimmune disease.

Results. The results of our studies are shown in the Table. Of the 9 patients, 7 were female. The average age at the time of presentation was 50 years. The average duration of lithium therapy was 59 months (range, 6 months to 15 years). Four of the 9 patients had either a personal or a family history suggestive of autoimmune thyroid disease or other autoimmune disorder. One other patient who had no personal or family history suggesting autoimmune disorder had antithyroid antibodies. All 9 patients had goiters and 8 had eye findings. Seven of the 9 patients had clinical Graves' disease; one had a toxic multinodular goiter and another had a solitary toxic nodule.

Discussion. Patients receiving lithium therapy for psychiatric disorders have been reported to have a variety of associated thyroid disorders, including hypothyroidism, autoimmune thyroiditis, hyperthyroidism, nontoxic goiter, solitary benign adenoma, papillary carcinoma, and endocrine exophthalmos. The most common lithium-associated disorder is hypothyroidism, which has been estimated to occur in between 1% and 25% of patients on lithium therapy (22, 23). The currently accepted incidence is approximately 5% (2). Patients with preexisting autoimmune thyroiditis appear to be at greatest risk for developing hypothyroidism while on lithium therapy (24). The hypothyroidism occurs as a result of inhibition of release of thyroid hormones from the

---

From the Division of Endocrinology, Department of Medicine, Mount Sinai School of Medicine. Address reprint requests to David K. Sirota, M.D., Division of Endocrinology, Box 1055, Mount Sinai School of Medicine, New York, NY 10029.

TABLE

Clinical Findings

Case No.	Age/Sex	Duration Lithium Rx	PHx or FHx thyroid or autoimmune disease	Antithyroid antibodies and TSI	Physical findings
1	43/F	5 yrs	none	not obtained	goiter eye signs
2	41/F	4 yrs	FHx diabetes mellitus	antibodies − TRAb −	goiter eye signs
3	47/F	9 mos	none	Antibodies − TRAb −	goiter eye signs
4	58/F	4 yrs	Mother: "thyroid condition" FHx multinodular goiter	Antibodies + TRAb +	goiter eye signs
5	46/F	6 mos	Sister: thyroid disease	not obtained	goiter eye signs tremor
6	40/M	4 yrs	none	Antibodies +	goiter eye signs tremor
7	70/F	2 yrs	none	Antibodies − TRAb −	multinodular goiter stare
8	65/M	15 yrs	none	Antibodies − TRAb −	toxic nodule
9	38/F	9 yrs	Mother: hyperthyroid	Antibodies − TRAb +	goiter eye signs tremor

+, positive; −, negative.

thyroid gland by lithium (2). This effect may be mediated by interference with microtubular function (2). Lithium is also known to reduce the radioactive iodine uptake in some species of laboratory animals (25). It has no effect on deiodinating activity in vitro, but it does prolong the half-life of labelled T_4 in peripheral tissue (26). The hypothyroidism associated with lithium therapy in human subjects is associated with the presence of elevated antithyroglobulin antibodies in a large number of patients, suggesting that lithium may accelerate the progression of autoimmune thyroiditis (27). Other data suggest that lithium may induce the formation of the antithyroglobulin antibodies directly (2).

Although hyperthyroidism has been associated with lithium therapy, there is no evidence that lithium is causative. It has been suggested that lithium-associated thyrotoxicosis is a result of overcompensation in response to abnormalities in iodine kinetics caused by lithium (3, 28), or alternatively that lithium causes thyrotoxicosis indirectly by its effects on intrathyroidal iodine (29).

One of the 9 patients reported on here developed hyperthyroidism shortly after reducing her lithium dose. This type of onset has also been reported in the literature in four additional patients (9, 14, 17, 21). This suggests that, in this group of patients, lithium may have been acting as an antithyroid agent, controlling underlying hyperthyroidism, which was then unmasked when lithium was discontinued. In this regard, it is interesting that in the present series the average age of the patients at the time of onset of hyperthyroidism was 50 years. This is somewhat higher than the age at onset of hyperthyroidism reported for the population not treated with lithium (30), again suggesting that lithium may be delaying the development of overt hyperthyroidism by its antithyroid properties.

The reported incidence of bipolar depressive illness is approximately 300 per 100,000, or 0.3%. Perhaps 600,000 patients with bipolar depressive illness are under treatment in the United States; a majority has been treated with lithium (31). Thyrotoxicosis has an incidence of 19 per 100,000 (32). Based on these data, an incidence of thyrotoxicosis among patients on lithium therapy of approximately 150 patients per year could be expected. In fact, including the 9 patients reported here, only 34 cases of thyrotoxicosis have been reported among patients receiving lithium therapy since its use in the treatment of emotional disorders was begun in 1949.

We conclude that lithium does not cause hyperthyroidism. Hyperthyroidism occurring in lithium-treated patients appears to be a chance phenomenon.

References

1. Schou M, Amidson A, Eskjaer S, Olsen T. Occurrence of goiter during lithium therapy. Br Med J 1968; 3:710–713.
2. Lazarus JH. Endocrine and metabolic effects of lithium. New York: Plenum, 1986:208.
3. Brownlie BEW, Chambers ST, Sandler WA, Donald RA. Lithium associated thyroid disease: a report of 14 cases of hypothyroidism and 4 cases of hyperthyroidism. Aust NZ J Med 1976; 6:223–229.
4. Lindstedt G, Nilsson L-A, Wallnder J, Skott A, Ohman R. On the prevalence, diagnosis and management of lithium-induced hypothyroidism in psychiatric patients. Br J Psychiatry 1977; 130:452–458.
5. Amidsen A, Anderson CT. Lithium treatment and thyroid function. Pharmacopsychiatria 1982; 15;145–155.
6. Brownlie BEW, Turner JG, Millner GMB, Perry EG, Ding L. Lithium associated thyroid cancer. Aust NZ J Med 1980; 10:62–63.
7. Segal RL, Rosenblatt S, Eliasoph I. Endocrine exophthalmos during lithium therapy of manic-depressive disease. N Engl J Med 1973; 289:136–138.
8. Franklin LM. Thyrotoxicosis developing during lithium treatment: case report. NZ Med J 1974; 79:782.
9. Rosser R. Thyrotoxicosis and lithium. Br J Psychiatry 1976; 128:61–66.
10. Bafageeu HH, Myers DH. Lithium and thyrotoxicosis. Lancet 1976, 1:1409.
11. Cubit T. Lithium and thyrotoxicosis. Lancet 1976; 1:1247–1248.
12. Merry J. Lithium and thyrotoxicosis. Br Med J 1977; 2:765.
13. MacGregor G. Hyperthyroidism and a parathyroid adenoma complicating lithium treatment. Lancet 1977; 11:1129–1130.
14. Reus VI, Gold P, Post R. Lithium-induced thyrotoxicosis. Am J Psychiatry 1979; 138:724–725.
15. Chaudhri MA, Bebbington PE. Lithium therapy and ophthalmic Graves' disease. Br J Psychiatry 1977; 130:420.
16. Pallisgaard G, Frederiksen PK. Thyrotoxicosis in a patient treated with lithium carbonate for mental disease. Acta Med Scand 1978; 204:141–143.
17. Schoenberg M, Ts'o TOT, Meisel AN. Graves' disease manifesting after maintenance lithium. J Nerv Ment Dis 1979; 167:575–577.
18. Rabin PL, Evans DC. Exophthalmos and elevated thyroxine levels in association with lithium therapy. J Clin Psychiatry 1981; 42:398–400.
19. Valenta LJ, Elias AN, Weber DJ. Hyperthyroidism and propyl-thiouracil-induced agranulocytosis during chronic lithium carbonate therapy. Am J Psychiatry 1981; 138:1605–1607.
20. Todd J, Jerram TC. Thyrotoxicosis during lithium treatment. Br J Clin Pract 1978; 32:201–203.
21. Thompson CJ, Baylis PH. Asymptomatic Graves' disease during lithium therapy. Postgrad Med J 1986; 62:295–296.
22. McDermott MT, Burman KD, Hofeldt FD, Kidd GS. Lithium associated thyrotoxicosis. Am J Med 1986; 80:1245–1248.
23. Transbol I, Christiansen C, Baastrup PC. Endocrine effects of lithium: 1. Hypothyroidism: its prevalence in long-term treated patients. Acta Endocrinol 1978; 87:759–767.
24. Edhag O, Swahn A, Webster PO. Hypothyroidism following lithium treatment. Acta Med Scand 1973; 193:553–555.
25. Berens SC, Bernstein RS, Robbins J. Antithyroid effects of lithium. J Clin Invest 1970; 49:1357–1367.
26. Mannisto PT. Effect of lithium on deiodinating activity of various rat tissues in vitro. Acta Endocrinol 1974; 76:260–272.
27. Edhag O, Swahn A, Webster PO. Hypothyroidism following lithium treatment. Acta Med Scand 1973; 193:553–555.
28. Kristensen O, Harrestrup Andersen H, Pallisgaard G. Lithium carbonate in the treatment of thyrotoxicosis: a controlled trial. Lancet 1976; 1:603–605.
29. Fradkin J, Wolff J. Iodine-induced thyrotoxicosis. Medicine (Baltimore) 1983; 62:1–20.
30. Turnbridge WMG, Evered DC, Hall R, et al. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol 1977; 7:481–493.
31. Berger PA. Medical treatment of mental illness. Science 1978; 200:974–981.
32. Furszyfer J, Kurland LT, McConahey WM, Elveback LR. Graves' disease in Olmsted County, Minnesota, 1935 through 1967. Mayo Clin Proc 1970; 43:636–644.

Submitted for publication January 1991.

Ovarian Pregnancy with Ipsilateral Mature Cystic Teratoma of the Ovary:

Case Report

MELISSA FELTINGOFF, DEBRA S. HELLER, M.D., AND IRA J. BLEIWEISS, M.D.

Abstract

Ovarian pregnancy is a rare form of ectopic gestation. Mature cystic teratoma is the most common form of germ cell tumor, and usually occurs in the reproductive age group. The concomitant occurrence of an ovarian pregnancy with a mature cystic teratoma in the same ovary is exceedingly rare. We were able to find two other such cases in the literature. We present a case, and discuss the entity.

OVARIAN PREGNANCY is a rare form of ectopic gestation which occurs at a frequency of one in 25,000 pregnancies and accounts for approximately 1% of all ectopic pregnancies (1). Mature cystic teratoma is the most common type of germ cell neoplasm. It comprises 5%–25% of all ovarian tumors (2). The concomitant occurrence of an ovarian pregnancy with a mature cystic teratoma in the same ovary is a rare event. In a computerized literature search we could find only two other cases of concurrent ovarian pregnancy and mature cystic teratoma in the same ovary.

Case Report. The patient was a 43-year-old gravida 4, para 2012, whose last menstrual period was six weeks prior to admission. She complained of sharp, intermittent lower abdominal pain for three days, and light vaginal bleeding for six days. Her gynecologic history was significant for two normal spontaneous vaginal deliveries without complications, one elective abortion, and no history of pelvic inflammatory disease. Her medical and surgical histories were noncontributory.

On physical examination, her vital signs were stable. Her abdomen was soft, mildly distended, with normal bowel sounds. Bilateral lower quadrant tenderness with rebound was present. Her pelvic examination showed a moderate amount of blood in the vagina. Her cervix was open with tissue in the os. The uterus was seven weeks in size. No adnexal masses were palpated. Her laboratory results were significant for a hemoglobin of 10.3g/dl, a hematocrit of 29.1%, and a white blood cell count of $14{,}600/cc^3$. Her urine pregnancy test was positive. The remainder of the laboratory tests were within normal limits.

Her initial diagnosis was probable spontaneous incomplete abortion. Ultrasound was not performed. The patient was admitted as an emergency admission on a weekend, when ultrasound was not readily available. Although a sonogram might have revealed an adnexal mass which had not been palpated, it would not have changed the management in this case. The patient had a positive pregnancy test, an open cervical os with tissue protruding, and abdominal pain. The differential diagnosis was incomplete abortion vs. ectopic pregnancy.

Dilatation and curettage was performed. On frozen section, no chorionic villi were present, and laparoscopy was performed, disclosing a ruptured left ovarian cyst and hemoperitoneum. Laparotomy was then performed, and a ruptured left ovarian cyst with adhesions to the posterior uterus and sigmoid, left salpingeal edema, and a 200 cc

---

From the Lillian and Henry Stratton-Hans Popper Department of Pathology, The Mount Sinai Medical Center. Address correspondence to Debra Heller, M.D., Dept. of Pathology, Box 1194, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY, 10029.

hemoperitoneum were encountered. The uterus and right adnexum were unremarkable. A left salpingo-oophorectomy and lysis of adhesions were performed. The patient had an unremarkable recovery.

Pathologic Findings. The left fallopian tube was grossly edematous but otherwise unremarkable. The ovary measured 6.0 × 4.0 × 3.0 cm, with areas of hemorrhage and fibrinous exudate externally. The cut surface showed a cyst containing yellow material with hair, and a corpus luteum was grossly identifiable (Fig. 1).

Histologically, an ectopic gestation was present in the ovary, near the corpus luteum, although invasion of the corpus luteum by trophoblast could not be demonstrated. A typical mature cystic teratoma was also present in the left ovary, squamous epithelium, sebaceous glands, and adipose tissue being the predominant elements (Fig. 2). The left fallopian tube was histologically unremarkable.

Discussion. Saint Maurice in 1692 described the first case of an ovarian pregnancy (3). Spiegelberg originally detailed the requirements for diagnosis of an ovarian pregnancy (3, 4): (a) the tube must be intact and clearly separate from the ovary; (b) the gestation must be in the position of the ovary; (c) the pregnancy must be connected to the uterus by the ovarian ligament; and (d) ovarian tissue must be demonstrated within the gestational sac. The diagnosis of ovarian pregnancy can be technically difficult. All hemorrhagic lesions of the ovary should be meticulously handled and examined histologically in serial sections so that a diagnosis of ovarian pregnancy is not missed (1).

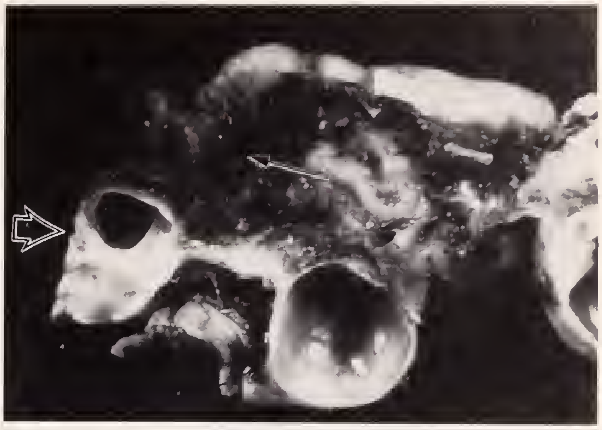


Fig. 1. Ovary appeared hemorrhagic at site of gestation (arrow), and cystic area containing yellow material and hair was present (arrowhead).

Mature cystic teratomas are more frequent occurrences. They are the most common germ cell tumor, occurring most commonly in the ovary but

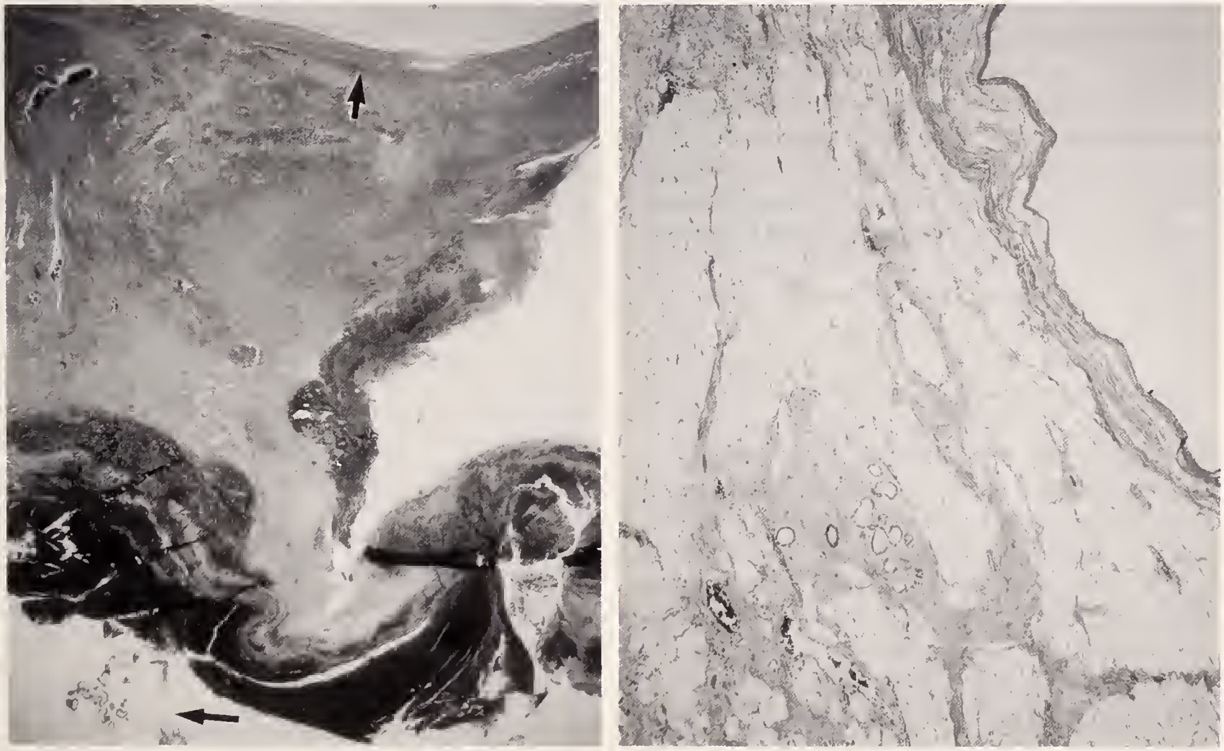


Fig. 2. Left Implantation site is seen near corpus luteum (arrow). Portion of mature cystic teratoma is also present (arrowhead), showing flattened squamous epithelium, and keratinaceous material in the cystic space. Right Typical mature cystic teratoma showing flattened squamous epithelium lining a cystic space, adipose tissue, and sweat glands (H&E, original magnification ×50).

may be found anywhere along the line of migration of the primordial germ cells from the yolk sac to the primitive gonad (1). Mature cystic teratomas usually occur during the reproductive years (4). They account for 22%–40% of ovarian tumors in pregnancy, and 0.8%–12.8% of reported cases of mature cystic teratoma have occurred during pregnancy (2).

Most cases of ovarian tumors that have been reported with a concurrent ectopic pregnancy have been associated with a tubal pregnancy (5–7). Luft reported that 67.3% of concomitant ovarian tumors and tubal pregnancies occurred on the same side (6). Three of his series of 49 ovarian tumors associated with tubal ectopic gestations were mature cystic teratomas. Alvarez described a mature cystic teratoma and ipsilateral tubal ectopic pregnancy. He concluded that since benign cystic teratomas are the most common ovarian tumors associated with pregnancy, they should also be the tumors most frequently associated with ectopic pregnancies (5). Massouda (7) reported on a case of benign cystic teratoma of the fallopian tube, a rare lesion in itself, associated with an ectopic gestation in the same tube.

We found two cases in the literature similar to the one we report here. In 1977 Gilardenghi described an ovarian pregnancy implanted at the hilum associated with an ipsilateral dermoid cyst (8). Yu in 1984 reported a case of an intrafollicular ovarian pregnancy in a mature cystic teratoma (3).

In summary, mature cystic teratomas are the most common type of ovarian tumor found during pregnancy. Although mature cystic teratomas are the most frequent tumor found in ectopic pregnancies, the incidence of ectopic pregnancies is 1 in 200 pregnancies; therefore the number of reported cases of dermoids associated with ectopic pregnancies is low. Ovarian pregnancies are a rare form of ectopic pregnancies. Thus the occurrence of a mature cystic teratoma and an ovarian pregnancy is an exceptional circumstance.

Acknowledgments

The authors thank Dr. Ruth Kreitzer and Dr. Renate Dische for their assistance with translation.

References

1. Bercovici B, Pfau A, Liban E. Primary ovarian pregnancy: report of three cases, one with implantation in an endometrial cyst of the ovary. Obstet Gynecol 1958; 12:596–600.
2. Turner RJ, Day PL. Torsed infected dermoid cyst with concurrent ectopic pregnancy. Mil Med 1986; 151:179–180.
3. Yu TJ, Iwasaki I, Teratani T, Tanaka T, Aoki M. Primary ovarian pregnancy in a cystic teratoma. Obstet Gynecol 1984; 64suppl:52s–54s.
4. Kurman R, ed. Blaustein's Pathology of the female genital tract, 3rd ed. New York: Springer-Verlag, 1987:422, 686–693.
5. Alvarez PA, Lopez AG, Mateu VS. Consideraciones sobre el embarazo ectópica: Revisión de 32 casos incluyendo uno de rotura tubárica derecha y quiste dermoide del mismo ovario. Acta Obstet Gin Hisp-Lus 1979; 6:397–414.
6. Luft H. Die bewertung der ovarialtumoren bei ektopischer schwangerschaft. Geburtshilfe und Frauenheilkunde 1970; 30:759–763.
7. Massouda D, Wortham GF, Oakly JL. Tubal pregnancy associated with a benign cystic teratoma of the fallopian tube: a case report. J Reprod Med 1988; 33:563–564.
8. Gilardenghi F. Un caso di gravidanza extrauterina su un ovaio con cisti dermoide. Min Gin 1977; 29:575–579.

Submitted for publication April 1991.
Final revision received September 1991.

Familial Adenomatous Polyposis:

Case Report and Review of Extracolonic Manifestations

Kenneth Alan Konsker, M.S.

A case of familial adenomatous polyposis is reported. A brief overview of that disorder, its extracolonic manifestations (periampullary carcinoma and desmoid tumors), and its possible association with multiple endocrine neoplasia are described.

Case Report

A 51-year-old Hispanic man from Peru consulted a physician for the evaluation of rectal pain.

The patient was in good health until one year before this visit, when he noted the onset of diarrhea that was "watery and greasy," averaging about four bowel movements per day. Three months prior to admission, the patient had hematochezia for one week, and seven days prior to admission he developed tenesmus. There were no precipitating or palliating factors associated with the rectal pain. The patient reported that he had no abdominal pain, hemorrhoids, recent travel, change in appetite, weight loss, nausea, vomiting, constipation, or fever and chills.

A rectal examination performed prior to admission showed adequate sphincter tone, diffuse tenderness, and a nodular mass posteriorly near the dentate line. The patient underwent a flexible sigmoidoscopy. At least 50 small polyps ranging from 2–7 mm in size were noted lining the rectum and rectosigmoid area. Biopsy revealed that they were tubular adenomas; two polypoid masses were also biopsied, one at the rectosigmoid junction and the other near the dentate line, and both were identified as fragments of adenocarcinoma. Esophagogastroduodenoscopy, performed to rule out upper gastrointestinal tract lesions, was normal. Colonoscopy was performed to the level of the transverse colon and revealed carpeting with hundreds of 3–7 mm polyps.

His medical and surgical histories were negative. Family history was significant in that the patient's mother died of "some type of colon problem at age 61." The patient has never been married, and no other family members were similarly affected.

On admission, the patient was a cachetic, well hydrated, alert man in no acute distress and appeared his stated age. The vital signs were as follows: blood pressure 120/80 mm Hg, pulse 72/min, respiratory rate 16, and temperature 98.2°F. The abdomen was soft, nontender, nondistended, and without organomegaly, mass, or ascites. Examination of the lymph nodes, especially of the inguinal area, was unremarkable. Positive physical findings were limited to the rectal examination, which showed a hard, immobile mass posteriorly; the stool was brown and contained occult blood.

Routine laboratory examinations showed a white blood cell count of 7800/mm^3, hemoglobin of 9.6 gm/dL, hematocrit of 30.4%, and mean corpuscular volume of 63.9 fL. Other findings of the hemogram, blood chemistries, and hepatic panel were normal. Platelets and PT/PTT were within normal limits. Findings on chest x-ray and flat and upright abdominal films were all negative. Urinalysis was unremarkable.

The patient underwent a total proctocolectomy with a Brooke ileostomy. There was an absence of metastatic lesions in the liver. Pathologic evaluation showed hundreds of small polyps and 10 large polyps (4 in the right colon, 1 in the

---

Written when the author was a third-year student at the Mount Sinai School of Medicine (CUNY). Address reprint requests to the author at 50 E. 98th Street, Apt 4K, New York, NY 10029.

transverse colon, and 5 in the left colon). The small polyps were tubular adenomas; the large polyps were villous tumors. Three of the villous tumors in the left colon showed adenocarcinoma without invasion. There was one flat, moderately differentiated adenocarcinoma in the anorectum which infiltrated the superficial layer of the muscularis propria. The 19 lymph nodes found in the specimen were free of tumor (Duke's B1 adenocarcinomas). The patient recovered uneventfully and was discharged on the fourteenth postoperative day.

Discussion

The first report of adenomatous polyposis of the colon was published in 1847 by Corvisart. In 1925, Lockhart-Mummery noted a high incidence of cancer of the colon in polyposis, particularly in younger persons with polyposis. In 1927, Cockayne showed that polyposis was inherited as a Mendelian dominant trait. In 1953, Gardner and Stephens described a syndrome comprising extracolonic manifestations of polyposis, including osteomas, and soft-tissue tumors such as lipomas, fibromas, and sebaceous cysts.

Familial adenomatous polyposis (FAP) occurs approximately once in 8000 births. The cardinal feature of this disease is the phenotypic expression of multiple adenomatous polyps throughout the lower gastrointestinal tract. Bussey defined FAP as "an inheritable condition in which the large intestine contains multiple adenomas, multiple being defined as more than 100" (1). The rectum is invariably involved. The adenomatous polyps generally appear in persons in their teens to their late twenties, and nearly all untreated patients die of large-bowel cancer before they reach 50 years of age.

FAP is an autosomal dominant disease with high penetrance, but about 20%–30% of patients do not have a perceivable genetic predisposition, and the disease is presumably the result of spontaneous genetic mutations. Rustin, in a retrospective review of the FAP registry at the Cleveland clinic, reported a 22% incidence of spontaneous mutations in family kindreds (2). It is highly likely that the patient I report on here had a spontaneous genetic mutation, since there is no family history of the disease. Also, his initial symptoms occurred when he was in his 50s, not when he was aged 20 to 30 years. The gene responsible for FAP has recently been mapped to a region on the long arm of chromosome 5 by polymorphic DNA markers (3).

The polyp-cancer sequence is greatly accelerated in this syndrome, even though the evidence of the adenoma–carcinoma sequence is circumstantial. Carcinomas occur in the same anatomic distribution as do the adenomas in the colon, and the risk for colorectal cancer is high in patients with a history of adenomas. If the adenomas are removed, the risk for progression to carcinoma is lowered. In the upper gastrointestinal tract, the distribution of adenomas parallels the sites for the risk of cancer, being found regularly in the duodenum and rarely in the stomach. Histologically, most of the adenomas are tubular; purely villous adenomas are rare. The larger the adenomas, the more likely they are to contain carcinoma.

The clinical course of an untreated or undiagnosed patient with FAP, as in the patient reported on here, is the development of colorectal adenomas in increasing numbers and then the inevitable development of colorectal cancer.

Diagnosis. Symptoms can be the harbinger of cancer. Normally the symptoms do not develop until there is a full-blown development of the polyposis. Bleeding from the rectum and diarrhea are the most common symptoms. Anemia may be present; microcytic anemia, probably due to blood loss, was evident in the case reported here. Bussey reported that two-thirds of patients with the symptoms of diarrhea, bleeding, and abdominal cramping had already developed carcinoma (1). The average age at the time of diagnosis of colorectal cancer in these patients is 39 years, compared to the normal population, where it is diagnosed between 67–69 years of age; clearly, this patient had atypical clinical features.

The polyps can be diagnosed by air-contrast barium enema, proctosigmoidoscopy, or colonoscopy. Diagnosis must be confirmed by the histologic findings of adenomatous polyps. Esophagogastroduodenoscopy and contrast studies with small-bowel follow-through are essential to rule out upper gastrointestinal polyps. Preoperative carcinoembryonic antigen values often correlate with tumor burden and prognosis, but the usefulness of this information is currently controversial.

Once the diagnosis of multiple adenomas in the colon is made, FAP should be differentiated from syndromes described by Gardner (4), Turcot (5), Cronkhite-Canada (6), Oldfield (7), and Muir (8). Gardner's syndrome is characterized by the triad of adenomas of the colon, osteomas, and soft-tissue tumors (lipomas, sebaceous cysts, fibromas). In Turcot's syndrome, adenomas of the colon are associated with medulloblastoma or glioblastoma. The Cronkhite-Canada syndrome is characterized by generalized intestinal polyposis in association with alopecia, cutaneous hyperpigmentation, and atrophy of the fingernails and toenails. In Oldfield's syndrome, multiple sebaceous cysts are associated with colonic intestinal polyposis, and in Muir's syndrome, multiple sebaceous cysts, laryngeal carcinoma, and duodenal carcinoma are associated with polyposis.

Evidence which blurs the distinction between these extracolonic expressions continues to accumulate, since combinations of phenotypic features formerly thought to be parts of separate disorders are being reported. Utsunomiya (9) and Bussey (10) report a growing body of evidence that Gardner's syndrome and FAP may not be separate entities but different expressions of a single, autosomal dominant, pleiotropic gene with variable penetrance.

Treatment. Because there is no effective conservative therapy, all patients with FAP should have a prophylactic colectomy to avoid the inevitable development of carcinoma. Proctocolecomy with a Brooke ileostomy remains the gold standard against which other alternatives must be compared. Because of persistent problems, including peristomal irritation, a restricted ability to pursue certain activities, and limitations in sexual activity, this procedure is not well accepted by young people. Most patients, given an alternative, seek to avoid this procedure but for the elderly or debilitated patient, a Brooke ileostomy remains the procedure of choice.

Proctocolectomy with a Kock's pouch, a popular option in the 1970s, is not substantially different from a Brooke ileostomy. This procedure abounds in cosmetic advances because there is no protruding nipple and an external appliance is not necessary. However, problems do exist: frequent extrusion of the nipple valve can result in incontinence and skin breakdown, and the stoma may leak and require revision in about 15% of the patients. Nevertheless, this procedure has proved to be effective in patients who cannot tolerate a Brooke ileostomy.

Abdominal colectomy with an ileorectal anastomosis is a third and attractive option. This popular procedure reduces the risk of development of cancer to only the last 15–20 cm of the rectum or rectosigmoid colon. Lifelong follow-up is necessary, and the patient needs to be compliant for such a program to be successful. The literature suggests that ileorectal anastomosis could be selected for those patients in whom the rectum contains few or no polyps, but since a lifelong follow-up is necessary, those patients also need to be reliable.

Controversy over the malignant potential of the rectum following ileorectal anastomosis persists in the literature. In the studies of Bess (11), Bussey (1), and Skinner (12), the subsequent development of cancer in the retained portion of the rectum ranged from 5% to 32% over approximately 20 years of follow-up. Factors that may account for the outcome differences are age of the patient at initial operation, length of the retained rectal segment, quality and frequency of sigmoidoscopic follow-up, and whether or not cancer was present in the resected colon.

Since 1980, the procedure of abdominal colectomy, mucosal proctectomy, and ileoanal anastomosis has revolutionized the surgical alternatives by combining many of the advantages of the other procedures. First, it completely eradicates all the colonic mucosa, thereby eliminating colonic manifestations of the disease and risk of malignancy. Second, normal or near-normal bowel function is preserved and a stoma is avoided. Bowel movements are generally reasonable, in that most patients have five or six movements daily with minimal urgency, and few if any at night. Lastly, their sexual function remains normal. This procedure is particularly attractive to individuals aged twenty to thirty years. There is the potential for major morbidity, such as pouchitis, pelvic sepsis, anastomotic breakdown, and bowel obstruction. But increasingly the ileoanal procedure appears to have particularly satisfactory results and has become the procedure of choice.

Extracolonic Manifestations

There has been much interest in the extracolonic expressions of FAP since its first description. The modern concept of FAP is that it is a genetically determined systemic disorder of growth regulation. The knowledge that periampullary carcinoma and desmoid tumors are more prevalent supports the notion that FAP is not a colonic disease alone. There are many known benign and malignant manifestations of this generalized growth disorder, including osteomas, congenital hypertrophy of the retinal pigment epithelium, gastric and duodenal polyps, desmoid tumors, epidermoid cysts, brain tumors, and periampullary, adrenal, and thyroid carcinomas. Sener et al., in a retrospective study of the extracolonic expressions in 141 patients with FAP, found that extracolonic manifestations were the rule rather than the exception; 89 of the 141 patients, or 63%, had one or more extracolonic expressions (13). Jagelman et al. reported that the risk for neoplasia increased beginning at the esophagogastric junction and extended distally throughout the entire mucosal epithelium of the gastrointestinal tract, particularly distal to the pylorus (14). The patient with FAP is susceptible to the formation of gastric polyps, but these are usually hamartomatous fundic gland polyps, not adenomas. To explain the differences in location and character of polyps and cancer, Jagelman et al. speculated that bile may act as a tumor promoter, and the pylorus may act as a barrier to reflux (14).

The worrisome complications of extracolonic manifestations are of two main sorts: upper gastrointestinal tract neoplasia and the consequences of abnormal fibroplasia. In a retrospective study at the Cleveland Clinic, Arvanitis et al. (15) found the major causes of death in patients with FAP who were cured of their propensity to develop colorectal cancer were periampullary cancers and desmoid tumors. Of the 110 patients who did not undergo prophylactic colectomy, the main causes of death were colorectal carcinoma (58%), desmoid tumors (11%), and periampullary carcinoma (8%). In contrast, the main causes of death in the 36 patients with FAP after prophylactic total proctocolectomy or total abdominal colectomy and ileorectal anastomosis were desmoid tumors (30.6%, occurring at an average of 6.6 years after surgery), periampullary cancer (22.2%, occurring at an average of 23.1 years after surgery), and rectal cancer (8.3%).

Periampullary Carcinoma. Because most reported cases involve late diagnosis, it has often been impossible to ascertain whether the cancer arose in duodenum, bile duct, or pancreas; hence the designation periampullary malignancy. In 1935, Cabot first described periampullary carcinoma in a patient with polyposis (16). Subsequent reports have strengthened this relationship, and Jones et al. suggested that the number of cases of this otherwise rare tumor in association with FAP has increased steadily (17). Duodenal polyps are invariably adenomatous, and upper gastrointestinal adenomas almost certainly predispose to malignancy. Bussey stated that endoscopists find 90% of patients with FAP have adenomas in the periampullary area and duodenum (10). Bulow et al. showed that biopsies of the duodenum in the region of the papilla of Vater should also be taken routinely in the absence of macroscopic lesions because villous changes in flat, apparently normal mucosa at that site were observed (18). Sarre reported that as family awareness and surveillance coupled with prophylactic colectomy is preventing death from colorectal cancer today, periampullary adenocarcinoma seems to be the most common malignant epithelial tumor occurring in these patients (19).

Longmire estimated that the incidence of periampullary malignancy in the general population was between .01% and .04%; the mean age of the patient with the malignancy was over 60 years (20). In contrast, Bussey's review of the St. Mark's Hospital experience showed an incidence of 4% for developing periampullary carcinoma in FAP patients and 12% in Gardner's syndrome patients who survived 5 years or more after colectomy (1). An increased risk for the development of periampullary cancer in FAP appears to occur in patients with a family history of periampullary neoplasia and possibly in patients with other stigmata of Gardner's syndrome.

The surgical approach to a small periampullary tumor is local excision: More extensive tumors are treated by a Whipple procedure. However, most patients, even under surveillance, develop inoperable tumors which can only be treated by bypass. Arvanitis et al. suggested a protocol whereby esophagogastroduodenoscopy and biopsy are performed annually in those patients who are found to have periampullary polyps, and every three years in those patients who have no polyps (15).

Desmoid Tumors. Desmoid tumors are a major life-threatening condition of polyposis patients. These tumors have been described pathologically as benign fibrous tumors occurring in the musculoaponeurotic soft tissues. Desmoids may occur either as a solid mass or as multiple whitish plaquelike lesions. Although they never metastasize, they are nonencapsulated and can grow to be quite large. Most desmoids occur in intraperitoneal or retroperitoneal locations. Klein et al. reported that because desmoids may grow and infiltrate surrounding viscera, causing pain, intestinal or ureteral obstruction, and fistulas, they constitute an important problem in the management of polyposis patients (21). These tumors can be felt by abdominal examination or noted on CT of the abdomen and pelvis.

Desmoids often arise in clusters in affected families. These tumors are relatively common in patients with FAP, although they are rare in the general population. Among 50,346 patients with neoplastic disease in the general population, Pack and Ehrlich encountered 17 patients (.03%) with desmoids (22). In contrast, Jones et al., in a survey of 325 FAP patients, demonstrated a 9% incidence of desmoids with a female-to-male ratio of

3:1 (23); most desmoids (86%) arose following prophylactic colectomy, and the majority were located intraabdominally, specifically within the mesentery of the small intestine.

Although the cause of desmoids is unknown, a number of risk factors and associated findings have been identified. Jones and Cornell (23) found that between 70% and 85% of patients with desmoid tumors are women 20–40 years of age; this increased incidence has been observed among parous women in the general population as well as in women with FAP. Another study (24) suggested that a history of prior trauma, particularly surgical, seemed to predispose one to the development of a desmoid in the vicinity of the surgery. Klemmer reported that a family history of desmoids also seemed to be a risk factor, both in the general population and in patients with polyposis syndromes (25). Clearly gender, hormone levels, prior surgery, familial history, and the presence of colorectal polyposis have all been implicated in the development of desmoids.

As confusing and intriguing as the etiology of desmoid may be today, even more perplexing is their effective treatment. The complete removal of the desmoid with no associated complications would be the ideal treatment. But desmoids are rarely small enough or sufficiently localized to allow resection of the tumor without sacrifice of vital structures, particularly the small intestine and its blood supply. Of 8 patients described by Jarvinen (26) who underwent surgery for the removal of their desmoids, 3 required major small-bowel resections; of the 3, 1 developed short bowel syndrome, and 2 required bypass.

In principle, operative resection should be avoided unless obstruction occurs or the tumors cause ischemia of the involved loops of small bowel. Unfortunately, not only is the attempted resection most often incomplete and associated with high morbidity, but the incomplete resection is always associated with a high recurrence rate. According to Klein et al., “Recurrence is the rule when desmoids are not completely removed” (21). Jagelman reported that recurrence of FAP-associated desmoids was so high that surgery is only used for specific complications, and any type of surgical approach seemed to exacerbate the growth of the desmoids (27).

Since the surgical treatment of desmoids is often so unsatisfactory, many have used a more conservative approach. The use of large doses of radiotherapy in treating desmoids, although often successful, remains controversial; it is associated with a number of complications, and its use in the management of intraabdominal desmoids is limited by the risk of intestinal and mesenteric radiation injury. Chemotherapy and other pharmacologic therapies, such as NSAIDs (indomethacin), hormonal treatment (Clinoril, tamoxifen, progesterone), and ascorbic acid have also been tried, with markedly inconsistent results. Further research is clearly needed in the development of a nonsurgical approach to the treatment of desmoid tumors.

FAP and MEN Syndrome. Endocrine neoplasms have been reported occasionally in patients with polyposis coli syndromes. Schneider et al. (28) reported the autopsy findings in 4 consecutive patients with polyposis syndromes in whom occult endocrine tumors similar to those in MEN Type I (pituitary, parathyroid, and pancreatic islet neoplasms) were discovered. Their review of 400 additional patients with colorectal cancer not associated with the polyposis syndromes revealed an incidence of occult tumors in only 11%

MEN Type IIB (medullary carcinoma of the thyroid, pheochromocytoma, multiple peripheral nerve tumors), like FAP, follows an autosomal dominant inheritance pattern, although approximately one-half of the MEN IIB and one-third of the FAP cases are sporadic. Perkins et al. described a patient who had Cushing’s syndrome and was found to have MEN IIB and adenomatous colonic polyposis with duodenal and gastric polyps (29). Although the authors stated that this relationship might have been coincidental, they suggested that there might be some common underlying mechanism involving genetic inheritance.

Nelkin et al. reported that the reduction to homozygosity for chromosome 10 regions, including those closely linked to MEN IIA and IIB, and sporadic medullary carcinoma of the thyroid was an infrequent event (30). Solomon et al. (31) and Volgenstein et al. (32) also showed a reduction to homozygosity in 20%–36% of the neoplasms in patients with familial polyposis. Bodmer et al. (33) and Vogelstein et al. (32) hypothesized that the first germ line chromosomal abnormality may have phenotypic consequences such as hyperplastic growth. The second hit(s) can either be a further dominant mutation at the other allele of the germ line defect or elsewhere, or it could be a recessive mutation in another location in the genome.

At this time, it is difficult to establish a true association between FAP and the MEN syndromes because of the paucity of cases in which both diseases have manifested in the same patient. Future work must look at the multiple mechanisms which may underlie the process of neoplastic transformation.

Summary

FAP, if left untreated, results in colorectal cancer. Appropriate early surgical intervention is of utmost importance. With the reduction in mortality from colorectal carcinoma, an increasing number of patients with FAP need lifelong follow-up to screen for extracolonic manifestations. Today, the major causes of death in patients with FAP who are cured of colorectal cancer, or have had a colectomy before its development, are desmoid tumors and periampullary carcinomas.

In family members at risk, screening with flexible sigmoidoscopy should be initiated in adolescence; symptoms of diarrhea and rectal bleeding would warrant an even earlier examination. The upper gastrointestinal tract should be assessed endoscopically at the time the diagnosis of FAP is made. If any polyps are detected, a biopsy is essential. If no gastric or duodenal polyps are found, repeated examinations at 3–5 year intervals probably suffice in asymptomatic patients.

Surgically, colectomy will be necessary. It is usually deferred until late adolescence, when it is thought that the patient will be mature enough to handle the emotional aspects of the operation as well as the possible future morbidity due to the procedure. The presence or absence of rectal polyps as well as the site and depth of any invasive rectal carcinoma will determine the appropriate surgical procedure. Alternatives must be well understood by the physician and discussed carefully with the patient preoperatively.

Acknowledgments

The author wishes to acknowledge the help of Drs. Demetrius Pertsemlidis at The Mount Sinai Hospital and Jerome DeCosse at Cornell Medical School.

References

1. Bussey HJR. Familial polyposis coli: family studies, histopathology, differential diagnosis and results of treatment. Baltimore, MD: The Johns Hopkins University Press, 1975.
2. Rustin RB, Jagelman DG, McGannon E, et al. Spontaneous mutation in familial adenomatous polyposis. Dis Colon Rectum 1990; 33:52–55.
3. Leppert M, Dobbs M, Scambler P, et al. The gene for familial polyposis coli maps to the long arm of chromosome 5. Science 1987; 238:1411–3.
4. Gardner EJ, Richards SC. Multiple cutaneous and subcutaneous lesions occurring simultaneously with hereditary polyposis and osteomatosis. Am J Hum Genet 1953; 5:139–147.
5. Turcot J, Despres JP, Pierre F. Malignant tumors of the central nervous system associated with familial polyposis of the colon, report of two cases. Dis Colon Rectum 1959; 2:465–468.
6. Cronkhite LW, Canada WJ. Generalized gastrointestinal polyposis: an unusual syndrome of polyposis, pigmentation, alopecia and onychotrophia. N Engl J Med 1955; 252;1011–5.
7. Oldfield MC. The association of familial polyposis of the colon with multiple sebaceous cysts. Br J Surg 1954; 40:534–541.
8. Muir EG, Yates Bell AJ, Barlow KA. Multiple primary carcinoma of the colon, duodenum, and larynx associated with keratoacanthomata of the face. Br J Surg 1967; 54:191–5.
9. Utsunomiya J, Nakamura T. The occult osteomatous changes in the mandible in patients with familial polyposis coli. Br J Surg 1975; 62:45–51.
10. Bussey HJR. Familial polyposis coli and hepatocellular neoplasms. Hepatology 1990; 12:175–6.
11. Bess MA, Adson MA, Elveback LA, et al. Rectal cancer following colectomy for polyposis. Arch Surg 1980; 115:460–67.
12. Skinner MA, Tyler D, Branum GD, et al. Subtotal colectomy for familial polyposis. Arch Surg 1990; 125:621–24.
13. Sener SF, Miller HH, DeCosse JJ. The spectrum of polyposis. Surg Gynecol Obstet 1984; 159:525–32.
14. Jagelman DG, DeCosse JJ, Bussey HJR, and the Leeds Castle Polyposis Group. Upper gastrointestinal cancer in familial adenomatous polyposis. Lancet 1988; 1:1149–51.
15. Arvanitis ML, Jagelman DG, Fazio VW, et al. Mortality in patients with familial adenomatous polyposis. Dis Colon Rectum 1990; 33:639–642.
16. Cabot RC. Case records of the Massachusetts General Hospital: case 21061. N Engl J Med 1935; 212:263–7.
17. Jones TR, Nance FC. Periampullary malignancy in Gardner's syndrome. Ann Surg 1977; 185:565–73
18. Bulow S, Lauitsen KB, Johansen AA, et al. Gastroduodenal polyps in familial polyposis coli. Dis Colon Rectum 1985; 28:90–3.
19. Sarre RG, Frost AG, Jagelman DG, et al. Gastric and duodenal polyps in familial adenomatous polyposis: a prospective study of the nature and prevalence of upper gastrointestinal polyps. Gut 1987; 28:306–14.
20. Longmire WP Jr. Periampullary tumors. J R Coll Surg Edinb 1973; 18:131–36.
21. Klein WA, Miller HH, Anderson M, et al. The use of indomethacin, sulindac, and tamoxifen for the treatment of desmoid tumors associated with familial polyposis. Cancer 1987; 60:2863–68.
22. Pack GT, Ehrlich HE. Neoplasms of the anterior abdominal wall with special considerations of desmoid tumors. Int Abstr Surg 1944; 79:177–198.
23. Jones EL, Cornell WP. Gardner's syndrome: review of the literature and report on a family. Arch Surg 1966; 92:287–300.
24. Jones IT, Jagelman DG, Fazio VW, et al. Desmoid tumors in familial polyposis coli. Ann Surg 1986; 204:94–97.
25. Klemmer S, Pascoe L, DeCosse J. Occurrence of desmoids in patients with familial adenomatous polyposis of the colon. Am J Med Genet 1987; 28:385–92.
26. Jarvinen HJ. Desmoid disease as part of familial adenomatous polyposis coli. Acta Chir Scand 1987; 153:379–83.
27. Jagelman DG. Extracolonic manifestations of familial polyposis coli. Semin Surg Oncol 1987; 3:88–91.
28. Schneider NR, Cubilla AL, Chaganti RSK. Association of

endocrine neoplasia with multiple polyposis of the colon. Cancer 1983; 51:1171–75.

29. Perkins JT, Blackstone MO, Ridell RH. Adenomatous polyposis coli and multiple endocrine neoplasia type 2b: a pathogenetic relationship. Cancer 1985; 55:375–81.
30. Nelkin BD, Nakamura Y, White RW, et al. Low incidence of loss of chromosome 10 in sporadic hereditary human medullary thyroid carcinoma. Cancer Res 1989; 49:4114–19.
31. Solomon E, Voss R, Hall V, et al. Chromosome 5 allele loss in human colorectal carcinomas. Nature 1987; 328:616–19.
32. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal tumor development. N Engl J Med 1988; 319:525–32.
33. Bodmer WF, Bailey CJ, Bodmer J, et al. Localization of the gene for familial adenomatous polyposis on chromosome 5. Nature 1987; 328:614–16.

Submitted for publication July 1991.

A+
NEW INDICATION

ONLY ONE H_2-ANTAGONIST HEALS REFLUX ESOPHAGITIS AT DUODENAL ULCER DOSAGE. ONLY ONE.

Of all the H_2-receptor antagonists, only Axid heals and relieves reflux esophagitis at its standard duodenal ulcer dosage. Axid, **150** mg b.i.d., relieves heartburn in **86%** of patients after one day and **93%** after one week.[1]

AXID®
nizatidine
150 mg b.i.d.

ACID TESTED. PATIENT PROVEN.

1. Data on file, Lilly Research Laboratories. *See accompanying page for prescribing information.* © 1991, ELI LILLY AND COMPANY NZ-2947-B-249304

AXID®

nizatidine capsules

Brief Summary. **Consult the package insert for complete prescribing information.**

Indications and Usage: 1. *Active duodenal ulcer*–for up to 8 weeks of treatment at a dosage of 300 mg h.s. or 150 mg b.i.d. Most patients heal within 4 weeks.

2. *Maintenance therapy*–for healed duodenal ulcer patients at a dosage of 150 mg h.s. at bedtime. The consequences of therapy with Axid for longer than 1 year are not known.

3. *Gastroesophageal reflux disease (GERD)*–for up to 12 weeks of treatment of endoscopically diagnosed esophagitis, including erosive and ulcerative esophagitis, and associated heartburn at a dosage of 150 mg b.i.d

Contraindication: Known hypersensitivity to the drug. Because cross sensitivity in this class of compounds has been observed, H_2-receptor antagonists, including Axid, should not be administered to patients with a history of hypersensitivity to other H_2-receptor antagonists.

Precautions: *General*–1. Symptomatic response to nizatidine therapy does not preclude the presence of gastric malignancy.

2. Dosage should be reduced in patients with moderate to severe renal insufficiency

3. In patients with normal renal function and uncomplicated hepatic dysfunction, the disposition of nizatidine is similar to that in normal subjects.

Laboratory Tests–False-positive tests for urobilinogen with Multistix® may occur during therapy.

Drug Interactions–No interactions have been observed with theophylline, chlordiazepoxide, lorazepam, lidocaine, phenytoin, and warfarin. Axid does not inhibit the cytochrome P-450 enzyme system; therefore, drug interactions mediated by inhibition of hepatic metabolism are not expected to occur. In patients given very high doses (3,900 mg) of aspirin daily, increased serum salicylate levels were seen when nizatidine, 150 mg b.i.d., was administered concurrently

Carcinogenesis, Mutagenesis, Impairment of Fertility–A 2-year oral carcinogenicity study in rats with doses as high as 500 mg/kg/day (about 80 times the recommended daily therapeutic dose) showed no evidence of a carcinogenic effect. There was a dose-related increase in the density of enterochromaffin-like (ECL) cells in the gastric oxyntic mucosa. In a 2-year study in mice, there was no evidence of a carcinogenic effect in male mice, although hyperplastic nodules of the liver were increased in the high-dose males as compared with placebo. Female mice given the high dose of Axid (2,000 mg/kg/day, about 330 times the human dose) showed marginally statistically significant increases in hepatic carcinoma and hepatic nodular hyperplasia with no numerical increase seen in any of the other dose groups. The rate of hepatic carcinoma in the high-dose animals was within the historical control limits seen for the strain of mice used. The female mice were given a dose larger than the maximum tolerated dose, as indicated by excessive (30%) weight decrement as compared with concurrent controls and evidence of mild liver injury (transaminase elevations). The occurrence of a marginal finding at high dose only in animals given an excessive and somewhat hepatotoxic dose, with no evidence of a carcinogenic effect in rats, male mice, and female mice (given up to 360 mg/kg/day, about 60 times the human dose), and a negative mutagenicity battery are not considered evidence of a carcinogenic potential for Axid.

Axid was not mutagenic in a battery of tests performed to evaluate its potential genetic toxicity, including bacterial mutation tests, unscheduled DNA synthesis, sister chromatid exchange, mouse lymphoma assay, chromosome aberration tests, and a micronucleus test.

In a 2-generation, perinatal and postnatal fertility study in rats, doses of nizatidine up to 650 mg/kg/day produced no adverse effects on the reproductive performance of parental animals or their progeny

Pregnancy–Teratogenic Effects–Pregnancy Category C–Oral reproduction studies in rats at doses up to 300 times the human dose and in Dutch Belted rabbits at doses up to 55 times the human dose revealed no evidence of impaired fertility or teratogenic effect; but, at a dose equivalent to 300 times the human dose, treated rabbits had abortions, decreased number of live fetuses, and depressed fetal weights. On intravenous administration to pregnant New Zealand White rabbits, nizatidine at 20 mg/kg produced cardiac enlargement, coarctation of the aortic arch, and cutaneous edema in 1 fetus, and at 50 mg/kg, it produced ventricular anomaly, distended abdomen, spina bifida, hydrocephaly, and enlarged heart in 1 fetus. There are, however, no adequate and well-controlled studies in pregnant women. It is also not known whether nizatidine can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Nizatidine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers–Studies in lactating women have shown that 0.1% of an oral dose is secreted in human milk in proportion to plasma concentrations. Because of growth depression in pups reared by treated lactating rats, a decision should be made whether to discontinue nursing or the drug, taking into account the importance of the drug to the mother.

Pediatric Use–Safety and effectiveness in children have not been established

Use in Elderly Patients–Healing rates in elderly patients were similar to those in younger age groups as were the rates of adverse events and laboratory test abnormalities. Age alone may not be an important factor in the disposition of nizatidine Elderly patients may have reduced renal function.

Adverse Reactions: Worldwide, controlled clinical trials included over 6,000 patients given nizatidine in studies of varying durations. Placebo-controlled trials in the United States and Canada included over 2,600 patients given nizatidine and over 1,700 given placebo. Among the adverse events in these placebo-controlled trials, only anemia (0.2% vs 0%) and urticaria (0.5% vs 0.1%) were significantly more common in the nizatidine group. Of the adverse events that occurred at a frequency of 1% or more, there was no statistically significant difference between Axid and placebo in the incidence of any of these events (see package insert for complete information).

A variety of less common events were also reported; it was not possible to determine whether these were caused by nizatidine.

Hepatic–Hepatocellular injury (elevated liver enzyme tests or alkaline phosphatase) possibly or probably related to nizatidine occurred in some patients. In some cases, there was marked elevation (>500 IU/L) in SGOT or SGPT and, in a single instance, SGPT was >2,000 IU/L. The incidence of elevated liver enzymes overall and elevations of up to 3 times the upper limit of normal, however, did not significantly differ from that in placebo patients. All abnormalities were reversible after discontinuation of Axid. Since market introduction, hepatitis and jaundice have been reported. Rare cases of cholestatic or mixed hepatocellular and cholestatic injury with jaundice have been reported with reversal of the abnormalities after discontinuation of Axid.

Cardiovascular–In clinical pharmacology studies, short episodes of asymptomatic ventricular tachycardia occurred in 2 individuals administered Axid and in 3 untreated subjects.

CNS–Rare cases of reversible mental confusion have been reported.

Endocrine–Clinical pharmacology studies and controlled clinical trials showed no evidence of anti-androgenic activity due to nizatidine. Impotence and decreased libido were reported with similar frequency by patients on nizatidine and those on placebo. Gynecomastia has been reported rarely

Hematologic–Anemia was reported significantly more frequently in nizatidine than in placebo-treated patients. Fatal thrombocytopenia was reported in a patient treated with nizatidine and another H_2-receptor antagonist. This patient had previously experienced thrombocytopenia while taking other drugs. Rare cases of thrombocytopenic purpura have been reported.

Integumental–Urticaria was reported significantly more frequently in nizatidine- than in placebo-treated patients. Rash and exfoliative dermatitis were also reported.

Hypersensitivity–As with other H_2-receptor antagonists, rare cases of anaphylaxis following nizatidine administration have been reported. Rare episodes of hypersensitivity reactions (eg, bronchospasm, laryngeal edema, rash, and eosinophilia) have been reported.

Other–Hyperuricemia unassociated with gout or nephrolithiasis was reported. Eosinophilia, fever, and nausea related to nizatidine have been reported.

Overdosage: Overdoses of Axid have been reported rarely. If overdosage occurs, activated charcoal, emesis, or lavage should be considered along with clinical monitoring and supportive therapy. The ability of hemodialysis to remove nizatidine from the body has not been conclusively demonstrated; however, due to its large volume of distribution, nizatidine is not expected to be efficiently removed from the body by this method.

PV 2093 AMP [101591]

Additional information available to the profession on request

Lilly

Eli Lilly and Company
Indianapolis, Indiana
46285

NZ-2947-B-249304

© 1991, ELI LILLY AND COMPANY

When you don't know all the medicines your patients are taking, protecting their health is a crap shoot.

Counseling patients about all their medicines improves their odds of getting well and staying well.

EVERYONE WINS WHEN YOU TALK

Send me a *free* Medicine Counseling Kit.

Name

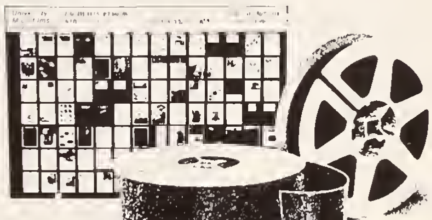
Organization

Street

City State Zip

Mail to:
National Council on Patient Information
and Education
666 Eleventh Street, NW, Suite 810
Washington, DC 20001
To fax your request -- (202) 638-0773

this publication is available in microform



Please send me additional information.

University Microfilms International

300 North Zeeb Road
Dept. P.R.
Ann Arbor, MI 48106
U.S.A.

18 Bedford Row
Dept. P.R.
London, WC1R 4EJ
England

Name

Institution

Street

City

State Zip

Instructions for Authors

THE MOUNT SINAI JOURNAL OF MEDICINE, established in 1934 as *The Journal of the Mount Sinai Hospital,* is a peer-reviewed general medical journal. It is indexed or abstracted in *Index Medicus, Current Contents, Science Citation Index, Hospital Literature Index, International Nursing Index, Excerpta Medica, Chemical Abstracts, Biological Abstracts,* and major databases. Theoretical and basic science articles for the clinician, reviews, reports of clinical or research experience, articles on any subject affecting the practice of medicine, short notes in any specialty, book reviews, letters, and articles by medical students are welcome. The *Journal* is a participant in the Agreement on Uniform Requirements for Manuscripts Submitted to Biomedical Journals.

Communications. Address correspondence on manuscripts review to: Managing Editor, *The Mount Sinai Journal of Medicine,* Box 1094, 19 E. 98th Street, 4D, New York, NY 10029-6574. Phone: (212) 241-6108; FAX: (212) 722-6386.

Copyright. The publisher reserves copyright and renewal rights on all published material, and such material may not be published elsewhere without written permission of the publisher. When submitting a manuscript, include the following assignment of copyright in your cover letter: **In consideration of The Mount Sinai Journal of Medicine (MSJM) taking action and reviewing and editing my (our) submission, the author(s) undersigned hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership to The Mount Sinai Journal of Medicine in the event that such work is published by MSJM.** Each author must sign the statement.

Manuscript Preparation. Submit manuscripts in triplicate, including three sets of illustrations. Manuscripts must be typewritten, **double-spaced throughout (including references, legends, and tables),** on one side of 8½ × 11-in. white bond paper, with generous margins. Number pages consecutively. Begin a new page for title page, abstract, first page of text, references, each table, and legends. **Computer-generated typescripts** must be double-spaced and must be easy for editors and typesetters to read. **Computer tapes or disks:** consult managing editor.

Title Page. On the title page, on separate lines (double-spaced), include (1) title of article, phrased as concisely as possible; (2) name of each author, including first name and degree; (3) name and address of departments and institutions from which the work originated; (4) acknowledgments of sources of support; (5) name, place, and date of any conferences at which paper has been delivered; (6) the statement **Address reprint requests to . . . ,** giving full name and address, with zip code, of the appropriate author.

Abstract. Original articles should include an abstract, which should be a single paragraph not exceeding 150 words. State the purposes; basic approach or methods; main findings; principal conclusions. Emphasize new and important aspects. When an abstract is provided, a summary at the end is not necessary. Reviews and other articles without an abstract should conclude with a short summary.

Key Words. Supply 3 to 10 key words or phrases at the bottom of the title page, for use in indexing the article. Use Medical Subject Headings from *Index Medicus.*

Writing Style and Abbreviations. Authors should aim for conciseness and clarity without repetition. Avoid medical jargon, abbreviated phrasing, and obscure or newly coined abbreviations. Define all abbreviations on first use, except those for standard, universally recognized units of measurement and statistical terms. Spell out such terms as white blood cell, hematocrit, superior vena cava.

Units of Measurement. Use SI units, giving range or interval of normal values.

Editing. All papers will be edited to enhance conciseness and clarity without altering meaning and to insure conformity with journal style. The author is responsible for all statements in the article.

Illustrations. Photographs, line drawings, graphs, and charts should increase understanding of the text. Line drawings, graphs, and charts should be professionally prepared. All illustrations should be submitted in triplicate as sharp, high-contrast glossy prints. Photomicrographs must be 5⅛ in. wide. On the back of each print affix a gum label with the number of the figure (numbered consecutively in arabic numerals as cited in text), title of manuscript, name of senior author, and the word "top" with an arrow indicating top edge. Illustrations which are to appear together should be mounted accordingly as plates and should correspond to each other in size. Protect the prints by enclosing them in heavy cardboard; do not use paper clips. Photographs of patients can be published only if a copy of the permission is submitted with the photograph. Eliminate names of patients and hospital numbers from x-rays.

Legends. Legends should not duplicate the text. Double space. Number each legend to match the illustration it accompanies. Illustrations mounted as plates to appear together should be accompanied by a single legend identifying the separate elements by position ("upper left," and so on) or by letters. If letters are used in the legend, a corresponding letter must appear on the print itself. Identify all abbreviations. Indicate magnification and stain for photomicrographs.

Tables. Each table should be typed on a separate page, double spaced. Number tables consecutively as they appear in text, using arabic numerals ("Table 1"). Give each table a brief title (for example, "Effect of DMSO on Rats") and type it on a separate line. If abbreviations or symbols are used in the table, identify them in a footnote.

References. When citing references in your text, the first work cited is numbered 1, and succeeding references are numbered in sequence; enclose the citation number in parentheses and place it before any punctuation: **Cytomegalovirus (1), steroids (2), and recreational drugs (3) have all been implicated (4).** The reference list includes only works cited in the text, numbered in the order in which they are cited. Type, double spaced, following the general arrangement and punctuation style in the examples below (journal title abbreviations conform to *Index Medicus* style):

1. Nadelman RB, Wormser GP. A clinical approach to Lyme disease. Mt Sinai J Med 1990;57:144–156.

2. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. N Engl J Med 1991;324:424–428.

When a manuscript is accepted for publication, you will be asked to confirm the accuracy of all references.

Proofs. Proofs are sent to the corresponding author from the printer. Stylistic changes which do not alter meaning should not be made at this stage. Because of increased printing charges, the cost of excessive author's alterations beyond routine correction of typographical errors and major errors in data may be billed to the author. Proofs should be corrected and returned to the editorial office within 48 hours.

Reprint Orders. A reprint order form is sent to the corresponding author with proofs; return it to the editorial office.

THE
MOUNT SINAI JOURNAL
OF MEDICINE

Forthcoming:
Grand Rounds

Graves' Disease
Terry Davies, MD
Mount Sinai School of Medicine

Is There A Future for Physicians in America?
Uwe E. Reinhardt, MD
Princeton University

Osteoporosis: On the Verge of Rational Effective Therapy?
John Paul Bilezikian, MD
Columbia University College of
Physicians and Surgeons

Obscure Gastrointestinal Bleeding
Blair S. Lewis, MD
Mount Sinai School of Medicine

Adrenergic Receptors in Hypertension and Heart Failure
Clive Rosendorff, MD
University of the Witwatersrand Medical School

Current Concepts of Systemic Necrotizing Vasculitis
Lee D. Kaufman, MD, and Allen P. Kaplan, MD
SUNY-Stony Brook School of Medicine

Theme Issues

MARCH 1992

**Hormones, Metabolism, and Society:
In Honor of Rosalyn S. Yalow**
Editor: Eugene Straus

SEPTEMBER 1992

**Human Cancer:
From Precurable to Curable**
Editors: Max Sung and Takao Ohnuma

NOVEMBER 1992

**Community Medicine:
In Honor of Kurt Deuschle**
Editor: Alan Silver

MARCH 1993

**Controversies in Neurosurgery:
Cervical Spine Disorders**
Editors: Martin Savitz and Kalmon Post

3 4805 0325536 7

Available now:

Toward the Conquest of Pain
Allan P. Reed, editor
volume 58, no. 3, May 1991
84 pages **$15**

Issues in Medical Ethics: Surrogacy and Drug Use
Daniel A. Moros and Rosamond Rhodes, editors
volume 58, no. 5, October 1991
96 pages **$15**

New Concepts in Asthma
Zvi M. Marom, editor
volume 58, no. 6, November 1991
81 pages **$15**

Subscriptions for 1992: $60 individuals, U.S. and Canada; $65 all libraries; $70 individuals outside U.S. and Canada.

To order subscriptions or copies of back issues ($15 each), please send check, payable to The Mount Sinai Journal of Medicine, to the Journal at Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574; counter sales at Suite 4D, 19 E. 98th Street, New York, NY (phone: 212 241-6108; FAX: 212 722-6386).

OF THE CITY UNIVERSITY OF NEW YORK

THE MOUNT SINAI JOURNAL OF MEDICINE

LUME 59 NUMBER 2 MARCH 1992

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society

Eugene W. Straus, editor

From the Bronx Veterans Affairs Medical Center, New York

Veterans Affairs

BETH
ISRAEL
MEDICAL
CENTER

THE MOUNT SINAI JOURNAL OF MEDICINE

The Mount Sinai Journal of Medicine is published by The Mount Sinai Medical Center of New York and has the following affiliates: Beth Israel Medical Center, New York; Bronx Veterans Affairs Medical Center, New York; and Elmhurst Hospital Center, New York.

Editor-in-Chief

Sherman Kupfer, M.D.

Editor Emeritus

Lester R. Tuchman, M.D.

Associate Editors

Harriet S. Gilbert, M.D. Julius Wolf, M.D.

Managing Editor

Claire Sotnick

Business and Production Assistant

Claudia N. Melendez

Assistant Editors

Stephen G. Baum, M.D.
David H. Bechhofer, Ph.D.
Constanin A. Bona, M.D., Ph.D.
Edward J. Bottone, Ph.D.
Jurgen Brosius, Ph.D.
Lewis Burrows, M.D.
Joseph S. Eisenman, Ph.D.
Richard A. Frieden, M.D.
Steven Fruchtman, M.D.
Paul L. Gilbert, M.D.
James H. Godbold, Ph.D.
Richard S. Haber, M.D.
Noam Harpaz, M.D.
Dennis P. Healy, Ph.D.
Tomas Heimann, M.D.
Barry W. Jaffin, M.D.
Andrew S. Kaplan, D.D.S.
Samuel Kenan, M.D.
Suzanne Carter Kramer, M.Sc.
Mark G. Lebwohl, M.D.
Kenneth Lieberman, M.D.
Charles Lockwood, M.D.
Steven Markowitz, M.D.
Bernard Mehl, D.P.S.
Myron Miller, M.D.
Edward Raab, M.D.
Allan Reed, M.D.
Allan E. Rubenstein, M.D.
David B. Sachar, M.D.
Henry Sacks, M.D.
Robert Safirstein, M.D.
Ira Sanders, M.D.
Martin H. Savitz, M.D.
Clyde B. Schechter, M.D.
Michael Serby, M.D.
Phyllis Shaw, Ph.D.
George Silvay, M.D.
Barry D. Stimmel, M.D.
Nelson Stone, M.D.
Max Sung, M.D.
Rein Tideiksaar, Ph.D.
Richard P. Wedeen, M.D.
Michael F. Wesson, M.D.

Editorial Board

Barry Freedman, M.B.A.
Richard Gorlin, M.D.
Nathan Kase, M.D.
Panayotis G. Katsoyannis, Ph.D.
Charles K. McSherry, M.D.
Jack G. Rabinowitz, M.D.
John W. Rowe, M.D.
Alan L. Schiller, M.D.
Alan L. Silver, M.D.
Alvin S. Teirstein, M.D.
Rosalyn S. Yalow, Ph.D.

The Mount Sinai Journal of Medicine (ISSN No. 0027-2507; USPS 284-860) is published 6 times a year in January, March, May, September, October, and November in one indexed volume by the Committee on Medical Education and Publications (Owner), The Mount Sinai Hospital, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Subscription price: individuals, U.S. and Canada, $60 per year; all libraries, $65; individuals outside the U.S. and Canada, $70. Single copies, $15. New subscriptions begin with the first issue of the calendar year. Send notice of change of address 60 days before the change is effective. Second-class postage paid at New York, NY and at additional mailing offices. POSTMASTER: Send address changes to The Mount Sinai Journal of Medicine, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Copyright 1992 The Mount Sinai Hospital.

THE
MOUNT SINAI JOURNAL
OF MEDICINE

Volume 59
Number 2
March 1992

CONTENTS

FESTSCHRIFT FOR ROSALYN S. YALOW:
HORMONES, METABOLISM, AND SOCIETY

Introduction *Eugene W. Straus* 95

Reminiscences and Reflections *Rolf Luft, J. Edward Rall, Marcus A. Rothschild, Enoch Gordis, Seymour Glick, Gabriel Rosselin, and Johanna Pallotta* 97

SCIENCE AND SOCIETY

Research in a Hierarchy of Values *Seymour M. Glick* 102

Science and Social Policy *Enoch Gordis* 108

Neonatal Hypothyroidism in India *N. Kochupillai* 111

REGULATION OF GASTROINTESTINAL FUNCTIONS

Physiology and Pathophysiology of Gastrin *John H. Walsh* 117

The Brooklyn Gastrinomas *Eugene W. Straus and Jean-Pierre Raufman* 125

Studies of a Proposed Mechanism Regulating Parietal Cell Emptying *Goran Nilsson, Bengt Romell, Kinfe Girma, and Rein Seensalu* 129

The Brain-Gut Axis: A Model System to Study Gene Regulation *Robert J. Greenstein* 135

MITOCHONDRIAL DYSFUNCTION

Luft's Disease Revisited: Severe Hypermetabolism of Nonthyroid Origin with a Defect in the Maintenance of Mitochondrial Respiratory Control *Rolf Luft* 140

NOVEL PEPTIDES AND ENZYMES

Exendin Peptides *John Eng* 147

Identification of a Novel Reductase in Folate Metabolism *Sheldon P. Rothenberg, David Kyner, and Gabriel Solomon* 150

BIOSYNTHESIS AND METABOLISM

Albumin Metabolism: A Brief Review *Marcus A. Rothschild and Murray Oratz* 155

continued

Volume 59
Number 2
March 1992

CONTENTS *continued*

Human Eicosanoid Biosynthesis and Metabolism *Carlo Patrono, Giovanni Ciabattoni, and Paola Patrignani* 157

Coronary Artery Disease: Metabolic Risk Factors and Latent Disease in Individuals with Paraplegia *William A. Bauman, Ann M. Spungen, Mohummad Raza, Jeffrey Rothstein, Run-Lin Zhang, You-Gong Zhong, Masako Tsuruta, Rubina Shahidi, Richard N. Pierson, Jr., Jack Wang, and Stanley K. Gordon* 163

ORGAN RESPONSE AND CELL DEVELOPMENT

The Response of the Myocardium to Overload Stress *Sidney S. Schreiber* 170

Development of the Beta Cell *B. Bréant, C. Lavergne, A. Astesano, N. Ferrand, M. Asfari, C. Boissard, A. Anteunis, and G. Rosselin* 175

Illustrations:

Rosalyn S. Yalow

Receiving the Nobel Prize from the King of Sweden, 1977 96
Delivering the Nobel Lecture 115
At her desk 124
Holding a guinea pig used for immunization 134
At work in the laboratory 185
Preparing the "atomic cocktail," 1948 inside back cover

Index to Advertisers

Eli Lilly and Company page 186

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, And Society

Introduction

Eugene W. Straus, MD
Chief
Division of Digestive Diseases
Department of Medicine
State University of New York
Health Sciences Center
at Brooklyn

This issue of the *Journal* celebrates a great woman of science. We need no special occasion to honor Rosalyn Yalow, but her 70th birthday is a milestone which we chose to observe in a symposium held on October 11, 1991 at the Mount Sinai Medical Center in New York City. In the distinguished audience were her husband, Aaron Yalow; her son, Benjamin Yalow; Mrs. Solomon Berson; and Nathan Kase, dean of the Mount Sinai School of Medicine.

Rosalyn Sussman Yalow has received an exultation of prizes and awards. More importantly, her work changed everything in the area of endocrinology, and it has opened new vistas across virtually the entire spectrum of biomedical science. We appreciate this in the details of specific studies, and in the ramifications and dissemination of the concepts which they created.

Brought together here are those who grew up on Kingsbridge Road at the Bronx Veterans Affairs Medical Center, participants in the efforts to see and truly to represent some aspect of nature. They shared the moments of discovery and frustration, tragedy and joy. And having been so enriched they went off to create on their own.

To honor Mount Sinai's distinguished professor, who so richly deserves our respect, our gratitude, and our devotion, authors from all over the world here bring offerings for her, adapted from their presentations at the October 1991 symposium. This issue of the *Journal* is dedicated to her, and to the spirit of scientific fellowship which she began in the Bronx as a young physicist in 1950. We cannot do this without paying homage to her magnificent partner whose name she revered in her professorship, for whom she named her laboratory so that he would be remembered in all of her publications, and whom we revere along with her in the fullness of our devotion to the memory of Solomon A. Berson.

Together they led us to follow "the angels of our better natures." We now warm our hands at the fire which they built by presenting our ideas to our incomparable Ros.

Rosalyn S. Yalow A Brief Synopsis of Her Career

Rosalyn Sussman Yalow received an A.B. in Physics and Chemistry from Hunter College in 1941 and an M.S. (1942) and Ph.D. (1945) in Nuclear Physics from the University of Illinois. She has been the recipient of 49 honorary doctorates from universities in the United States and abroad, including Yeshiva University, Tel Aviv University, Bar-Ilan University, Spertus College of Judaica, and Technion-Israel Institute of Technology.

In 1977 she was awarded the Nobel Prize in Physiology or Medicine for the development of radioimmunoassay (RIA), a methodology used in thousands of laboratories around the world to measure hundreds of substances of biologic interest in blood and other body fluids. In 1988 Dr. Yalow received the National Medal of Science.

She joined the Bronx Veterans Administration Medical Center in 1947 and has been a VA Senior Medical Investigator since 1972. Currently she is the Solomon A. Berson Distinguished Professor-at-Large of the Mount Sinai School of Medicine, CUNY, and Emeritus Distinguished Professor-at-Large of the Albert Einstein College of Medicine, Yeshiva University of New York. She is a member of the National Academy of Sciences and of the American Academy of Arts and Sciences. In 1986 her laboratory was designated a Nuclear Historic Landmark by the American Nuclear Society.

Dr. Yalow has received over 50 awards and prizes, among them the First Veterans Administration William S. Middleton Award for Medical Research, the Albert Lasker Basic Medical Research Award, the Gairdner Foundation International Award, the Lilly Award and Banting Medal of the American Diabetes Association, the Koch Award of the Endocrine Society, the Van Slyke Award of the American Association of Clinical Chemistry, and the G. van Hevesy Nuclear Medicine Pioneer Award.

She has served as president of the Endocrine Society as well as a member of numerous advisory agencies and editorial boards.

She is married to Dr. Aaron Yalow and has two adult children, Benjamin and Elanna.

Rosalyn S. Yalow receiving the Nobel Prize from the King of Sweden, 1977.

Reminiscences and Reflections

At the scientific symposium on October 11, 1991 at the Mount Sinai Medical Center honoring Dr. Rosalyn S. Yalow, during the luncheon and the subsequent dinner at the Plaza Hotel, many participants expressed their thoughts on the influence of Yalow and Berson on biomedical investigation and on their individual careers. Because of space limitations, what follows are abstracts of some of the remarks submitted in written form.

One evening when Arturo Toscanini left La Scala in Milano, where he had been conducting *The Magic Flute,* and came out on the piazza, an organ-grinder was grinding his barrel-organ as usual. Toscanini, upset by the monotonous sound, went up to the man and roundly told him off about varying the speed of the handle and the tone of the instrument. Next evening, when Toscanini left the theater, the man was standing there again, and the same monotonous sound filled the piazza. Furious, Toscanini rushed up to him, but froze when he saw a big sign behind the man saying: “Pupil of the great Toscanini.”

Ros Yalow and Sol Berson were the Toscaninis of the field we call radioimmunoassay. Most others were, if not organ-grinders, followers; they appreciated Ros's discovery with Sol, and used it for their purposes. This is as it should be. But the glory of scientific discovery—one of the few areas where questions of conflict have no place—rests on Ros's shoulders.

I have heard Ros say on a couple of occasions that a phone call from me changed her life. It may have, but only superficially. Ros became a public figure, as have so many Nobel laureates. This is not so remarkable, taking into account that the Nobel prize is the only recognition in science which every member of the scientific community in the world knows by name, and which is well known also among nonscientists. Furthermore, the prize elevates its recipients to the uppermost rank of the scientific ultra-elite, that thin upper layer of the hierarchy which exhibits great influence and authority. The prize confers on the recipients legitimacy in many ventures—political, social, even ideological—most of which are not in keeping with Mr. Nobel's intentions. And let us not forget that Ros is one of the few women in the sciences who have been recognized by a Nobel prize. Therefore, in a superficial way, my phone call—or actually the prize which my phone call announced to her—may have changed Ros's everyday life. Ros has served well in public life, and will continue to do so for many years to come, with honesty and devotion.

The Nobel prize, according to Mr. Nobel's will, is given for “a discovery—which shall have conferred the greatest benefit on mankind.” In other words: it is the enduring quality and impact of the scientific achievement which places the scientist in the ranks of the scientific elite and in the consciousness of members of the scientific community. Here only the quality and endurance of the discovery counts, not public appearances. Ros's discovery with Solomon Berson changed the course of biomedical research in an unforgettable way. But the Nobel prize had no major impact on Ros's place among scientists, where Ros will remain a historic figure and her discovery a landmark—regardless of any prize.

Now Ros has reached retirement age, and I understand that she is closing down the shop. Most people, including academic people, look forward during the later part of their careers to the moment when they can spend most of their time with their hobbies, forgetting that, say, collecting stamps is a hobby only when your day is filled with ordinary duties; as a main occupation it becomes a burden.

The mere thought of Ros devoting her time to a hobby is absurd. She has other interests to nurture, among them the universal problems of nuclear radiation. For her “retirement” is just a balancing of her books and the turning of a page in a rich and successful career.

An old friend recently wrote me about his decision to withdraw from academic activities at the age of 82. He writes: "I will vacate my office, distribute most of my books, and start contemplating my navel." By the age of 70, or even 90, my dear Ros will still not even know where that navel is.

Behind all I have said about Ros, behind the facade of fame, behind all the luster, I see one of my best friends: a woman to turn to for advice in scientific and in personal matters; a person who has been a strong support for me and my wife in times of illness and distress; a loving and caring companion. For this we thank you.

In "The Municipal Gallery Revisited," a poem by another Laureate (1923), William Butler Yeats, that I have recited for Ros, Yeats visits the municipal gallery of Dublin. He passes along the walls where the portraits of those who meant so much for Ireland hang. Many were his friends, and he knows that one day his own portrait will hang there. He ends with the following lines:

> You that would judge me, do not judge alone
> This book or that, come to this hallowed place,
> Where my friends' portraits hang and look thereon
> Ireland's history in their lineaments trace;
> Think where man's glory begins and ends,
> And say my glory was I had such friends.

Rolf Luft, MD
Prof. Emeritus of Endocrinology
Karolinska Institutet, Stockholm

Ros has been one of my oldest and best friends since the early 1950s. At that time, Ros and Sol Berson were working on, among other things, some aspects of thyroid function. They worked on the use of radioactive iodine as a measure of thyroid function, and devised the thyroid iodine clearance test, which still—almost 40 years later—is the only test using radioactive iodine, which is a physiologically sound analysis. What they measure is the quantity of blood cleared of iodine by the thyroid per unit time. This is a valid physiologic parameter, whereas an uptake of iodine at one hour, twenty-four hours, or any other time period is a quite arbitrary figure and conceals half a dozen or so variables.

I well remember the early days in the 50s and 60s when Ros, Sol Berson, and I would always meet at the annual meeting of the Young Turks, at that time held in Atlantic City, and argue about life and science. (Incidentally, Ros is one of the few nonphysician members of the Association of American Physicians.) I particularly remember an exchange that took place after Sol Berson had just turned down a professorship at some unnamed university, and in an expansive mood said, "Well, I would have made a great deal more money, but it's only money after all." To which Ros added, "Actually, not very much money."

Beginning in 1962, when I became the scientific director of what was at that time the National Institute of Arthritis and Metabolic Diseases, (I won't take you through the transmogrifications of the name of this venerable institute), Sol Berson became a scientific counselor of the institute and attended the biennial meetings religiously, as did Ros when she became a scientific counselor somewhat later. Together they were scientific counselors at this institute from 1963 to 1983 and gave generously and invaluably of their time and incisive scientific wisdom.

More recently, Dr. Yalow has been interested in radiation and the uninformed outcry of the public about imagined dangers of low levels of radiation. Anything Dr. Yalow puts her mind to she studies in careful, if not exhaustive, detail. I well remember a meeting of a committee I chaired to establish a table which would calculate the probabilities of causation from any given dose of radiation. On this committee were a distinguished group of radiobiologists; Ros tackled one of them, quoted his paper to him, and essentially destroyed his arguments, because he had forgotten some of the data he had actually published some twenty years before. Anyone planning to argue with Rosalyn Yalow would be well advised to be properly prepared.

So I am delighted to acclaim a brilliant and courageous scientist and a great person. I am proud to claim her as a friend.

J. Edward Rall, MD, PhD
Senior Scientist
National Institute of
Diabetes, Digestive,
and Kidney Diseases, Bethesda MD

It is so easy to remember the events that shaped my professional activities. The start of a change in my life came in early 1954 when I walked into the office at the Radioisotope Science Laboratory at the Bronx Veterans Administration Hospital and was accepted to train with Rosalyn Yalow and Solomon Berson. Isotopes was the VA research, and many leaders of the past 40 years in medical research came from this small group.

Blood volume studies kept us busy. We had scintillation counters, but the p32 for the measurements of red blood cell volume were done in a beta counter. The containers for the small sample of blood were made from metal bottle tops before they were stamped. Albumin distribution studies were conducted in patients with splenomegaly, congestive heart failure, proteinuria, and we measured the rates of equilibration of the labeled red cells and albumin. We showed how the relationship of the large-vessel hematocrit value and the average total body hematocrit value could change in health and disease. We used large doses of thyroid to show that albumin metabolism could be increased.

All of this work changed my plan. I gave up a spot in private practice and made the decision to go full-time into research.

Yet the most exciting moment—the work with tagged insulin—was yet to come. Working with Ros and Sol and watching their minds work was stimulating. Arthur Bauman and I did early studies after Kitty Newerly labeled the insulin. Ros and Sol then developed these findings into the great work that followed. This was my beginning.

I have had a wonderful research career examining many aspects of albumin metabolism. Sol was a most special person, and the friendship with Arthur is a source of happiness to me. Without the guidance, help, warmth, and support of Ros over many years, this would not have been possible.

Marcus A. Rothschild, MD
Clin. Prof., Radiology
University of Miami
Consultant, Nuclear Medicine Service
VAMC Miami

It was a happy year for me at Ros and Sol's lab at the Bronx VA. It was in the middle of my medical residency at The Mount Sinai Hospital in 1958–9.

The atmosphere was unusual. Sol and Ros worked intensely in their back lab and office, but classical music was usually on the radio at the same time. Conversation was animated and free, with a good deal of humor tossed in. Sol and Ros were perfect complements: both of them of course brilliant and original, Sol being more likely to take off in all directions, and Ros applying the critical brakes and putting the powerful analytic engine into gear early so ideas could be brought rapidly to fruition. Ros, starting as a physicist, ended up knowing more physiology than most leading physiologists.

One aspect of Ros's and Sol's life that gave them pride was the nurturing of the younger investigator. All of us loved them, and we knew they cared about us. The standards they set for hard work, rigor, uncompromising regard for truth and logic, and concern for people have been a guide in my life and, I'm sure, in the lives of all who have worked with them.

Enoch Gordis, MD
Director
National Institute on
Alcohol Abuse and Alcoholism, Rockville MD

I still remember my first day in the laboratory, running around to keep up with Ros and Sol on their forays in and out of the nooks and crannies of the laboratory. A visitor from Belgium had already been there for several days. By about 1:30 pm I whispered to him, "When do they stop for lunch here?" I was famished and saw no signs of anyone eating or offering food. Little did I know about the stock of cookies in the inner sanctum that prevented hypoglycemia.

One of the lessons I learnt in the laboratory was that of impeccable integrity and honesty. I marvelled at the extraordinary efforts made to assign credit in the most meticulous fashion, not merely by year of publication, but by month of publication. In addition, an outstanding feature of working with both Sol and Ros is the credit and the public praise they gave their fellows. It was easy enough to overtly or even covertly take credit for joint efforts. Such acts would be easily believable and were often true. But we were always given more than our deserved share of the credit and the "glory," a lesson which has remained with me always as a guide.

We also learned to conserve public money. It was not hard for Ros and Sol to get almost unlimited amounts of research funding and to live "big." But we were given to understand that public moneys are to be treated with no less respect than one's own, and that success in research does not depend on fancy lab equipment or furniture.

We were a protected species. Anyone who dared attack us unfairly had Sol and Ros in the full array of their most aggressive stances to contend with—after we had defended ourselves to the best of our ability. Ros also occasionally had to protect Sol from all kinds of pests and difficult people. Sol had one great failing: he did not appreciate that most people were neither as smart nor as honest as he, so he was a trusting and giving soul, even to those who did not deserve such treatment. Ros, ever the rational, cool-headed scientist and with superior insight, was much better at evaluating people, and she occasionally brought Sol down to earth.

All in all, my three years at the laboratory were a fantastic experience, and not just scientifically; they provided a broadening and enriching experience in many different areas, far afield from medicine.

I can only say thank you, and wish Ros many many more healthy and productive years.

Seymour Glick, MD
Gussie Krupp Prof. of
Internal Med., Ben-Gurion Univ. of
the Negev, Beer Sheva, Israel

In 1961, as resident at the Hotel Dieu of Paris, I suggested a program for developing insulin assay. In those days emphasis was put on the fat pad assay, considered the best method since it was based on biological effect. When I read the papers of Ms. Yalow and Mr. Berson I was struck, and from that moment I felt like someone who has bought a ticket to the next town and finds himself flying to the stars. The importance of the discovery was obvious; what had been done with insulin could be applied to any regulatory substance present at low concentration in the organism. I spent ten months in their lab, from September 1965 to July 1966, which were among the most fruitful of my life.

I can trace back to my stay in the United States, in the laboratory of Ros and Sol, the origin of some principles that are obvious to me now:

- Follow your own idea without letting material problems stop you.
- Do not lose time doing something other people have already done.
- Be aware of and keep in mind the elements of your experiments that you hold for sure and those you are not certain about.
- Respect competence first, whether it is or is not related to a preestablished hierarchy.
- Be cautious about spreading information on work performed in a lab as long as it is not finished.
- Take criticism into account, but do not wait to publish a paper, so long as you are confident of your own data.
- Be aware of charlatanism and treachery in science, to be able to fight it.

Gabriel Rosselin, MD
Director
Centre de Recherches
Paris Saint Antoine

I am flattered to participate in this celebration for my greatly esteemed former teacher, guide, and friend, Dr. Rosalyn Yalow.

At the end of my senior residency, when I went down to Drs. Berson's and Yalow's laboratory at the Bronx Veteran's Hospital, I had no inkling of what it would mean to me. It was Dr. Yalow who kindly convinced an understandably skeptical Dr. Berson that a mere senior resident physician who had vague ideas about "going into cardiology" (as I foolishly said) was a likely bet for a fellowship in nuclear medicine and radioimmunoassay. What I loved was the very human and totally upfront way she did it. "C'mon, Sol," she chided, "Give her a chance. She's *very* young." I was. He did. And it changed my life, for the good, and permanently. Drs. Berson and Yalow opened my eyes to a tremendously exciting field—endocrinology, which was being reinvented by their work—and it became my life's work. I am still bringing to the bedside of afflicted patients the results of the enormously helpful measurements and techniques I first learned to make and use with them.

Both were naturally great teachers. They knew what they were after, they knew how to pursue it, and they knew that it was of potentially immense importance. All I knew was that theirs was a tremendously exciting work environment.

Even though I am not likely to follow Dr. Yalow in the ranks of Nobel laureates, I must pay tribute to her as a superb role model. She has shown a way of being a great scientific-medical worker and, among other things, a very good mother and family woman. She was always "on call" for her family. She could have lived in a plush suburb, but then she wouldn't have been able to go home for lunch with her children, as she did many times. When my daughter Jackie was born some years after my time at the Bronx, Dr. Yalow flew to Boston and insisted I get live-in help, which she helped me do, so that I could go on with my career. She showed me that indeed there is no contradiction between having a full-time profession and being a real mother.

Once Dr. Yalow said to a *New York Times* interviewer, "I have respect for people only because of the qualities they have, not because of their position." On just those terms I am glad to offer my small tribute to her. May she and her family continue to flourish happily.

Johanna Pallotta, MD
Asst Prof., Medicine
Harvard Medical School—
Beth Israel Hospital, Boston

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society

Science and Society

Research in a Hierarchy of Values

SEYMOUR M. GLICK, M.D.

Abstract

Research fraud has unfortunately become increasingly troubling and public, undermining public confidence and support of research and threatening the foundation of research. The response of the scientific establishment has varied, but the public perception of the response is that it has been too little and too late. Science, like other institutions supported by public funds, must be accountable to the public and must accept public scrutiny without an overly defensive posture. Cooperation with such public review ultimately benefits science. The root problems underlying fraud in research are not unique to science, but reflect individual and societal moral weaknesses. Science and research, although essential and critical societal activities, do not represent the highest priorities of society, and must be subordinate to higher moral and ethical standards whose sources are external to science itself.

THIS IS an unusual opportunity to reflect a bit about research, its status, its problems, and its challenges. We gather here to honor a woman, Ros Yalow, whose whole professional life personifies research, who has devoted herself uncompromisingly and passionately for half a century to this love of hers, and who has achieved the highest honors possible in the field. Her stepping down represents in a way the end of an era, for we frequently hear more and more that modern science should be based on a model different from that exemplified by Ros and her late partner, Sol Berson: No longer the small laboratory, the personal touch, the hands-on experience, the one or two fellows, but rather the research "factory," with a leader who often is more at home in the boardroom than in the cold room. Indeed, many of science's major achievements, as well as its tragic shortcomings, may be rooted in this new model. This occasion is an appropriate one for reflection on research, its ambience, its ethos, and its appropriate place in our scale of values.

Scientific Fraud and the Response to It. The research establishment seems to be under attack, judging at least by the media, by presidential addresses to major national scientific bodies (1, 2), by editorials in scientific publications (3–5), or by special symposia (6, 7) devoted to the problems of research. Particularly highlighted recently has been the problem of fraud in research, which has become increasingly public and troubling, although not necessarily more frequent, over the past decade and a half. A news item in *Science* early in 1991 (8) began with the sentence, "Fraud is a growth industry, if a spate of conferences on the topic . . . is a fair index of what's to come in 1991" (1). Science can survive a variety of faults—irrelevance, wastefulness, arrogance, cruelty—without fatal damage. But an attack on the integrity of science undermines the foundations of science and essentially destroys it. Bentley Glass, in his John Calvin McNair lectures on the ethical basis of science (9), lists as the first commandment, complete truthfulness. That this quality is essential for science is indisputable; indeed, it has generally been taken for granted. In a 1985 lecture at the Moscow State University (10), Gerard Piel pointed out, "It is not that scientists are more dedicated, honest and selfless than other

---

From the Center for Medical Education, Jakobovits Center for Jewish Medical Ethics, Faculty of Health Sciences, Ben-Gurion University of the Negev, and the Division of Internal Medicine, Soroka University Hospital of the General Sick Fund, Beer Sheva, Israel. Address reprint requests to the author, Gussie Krupp Prof. of Internal Med., Ben-Gurion Univ. of the Negev, P.O.B. 653, Beer Sheva, 84-105, Israel.

citizens; they are disciplined to behave that way by their collaboration. Error and fraud are exposed sooner or later by the communal process of verification."

The increasingly frequent and vocal attacks on the integrity of science and its practitioners because of suspected dishonesty have elicited a variety of reactions. Public response varies from that of the legendary little boy who so sadly expressed his disappointment at the infamous Black Sox baseball bribe scandal by pleading with his player-idol, "Say it ain't so, Joe," to that of a host of individuals and groups who have seized this opportunity to attack science and its institutions, trying to restrict them and reduce their power, prestige, and independence. Anti-intellectualism and political opportunism underlie some, but far from all, of the vocal attacks on scientists and scientific institutions.

The response of our scientific establishment varies as well, and is far from monolithic. In its worst form it resembles the response of a man asked to return the lawnmower he had borrowed from a neighbor: "First of all I never borrowed it; second, I returned it; and third I need it for a few more days." At our best we have made vigorous attempts to police our own bailiwicks. But our attempts to clean house clearly have not fully convinced public officials, legislators, and the media that we are capable of self-policing. It is essential that, in our self-examination, we should not be defensive about the research endeavor itself. The benefits to humankind are so evident, even in proportion to the money spent, that we can face the world with more than a clear conscience. Yet what is so obvious to us may not be at all apparent to the public. And many competing interests in the community may need to be convinced of our point of view. Only a few short years ago most researchers confronted the animal rights movement with complacency; we were certain that the benefits of animal experimentation were so obvious as not to need repeating. We are now running scared, and appropriately so.

Yet our defense of research must be accompanied by rigorous and merciless self-examination, so that we can find and correct whatever defects exist in our own establishment. This kind of critical self-examination characterized the Berson Laboratory from its inception. Many who heard Ros or Sol at national meetings and sometimes cringed at their devastating critiques of papers may not have realized that Ros and Sol, in the privacy of their laboratory, were even more critical of their own results; no favor was shown to home-grown data. So too it is incumbent on us to examine not only our data, but our entire scientific establishment.

Fraud as a Human Failing. Research fraud is probably coeval with research, just as all human foibles date back to the appearance of *Homo sapiens* on earth. Cain and Abel, who had available the whole world and its resources, yet could not bear sharing the wealth and the limelight equally, were not unique, but were paradigmatic of our human enterprise. Research fraud is not new, and it will be with us as long as men and women, and not angels, do research. In her 1984 presidential address to the Central Society for Clinical Research (2), Virginia Donaldson, with tongue-in-cheek female chauvinism, pointed out that the list of fraudulent scientists she had assembled included no women—but predicted, correctly, that given time, women too will achieve such note.

Fraud and deception are inherent in man's and woman's potential, as are integrity and honesty. The list of known wrongdoers in the hall of fame of science since time immemorial includes some of the world's leading scientists, who on occasion were guilty of overt fraud, plagiarism, and battles for priority, activities described in detail by many historians and sociologists of science (11).

Scientists are human. This humanity is the basis for the successes of scientists and for their failures. Scientists bring to the laboratory bench not only their knowledge and skills, but also their personality and set of values. Ros is not only a scientist but also a Jewish mother—a much maligned species of humanity. One of my sociologist friends recently brought to my attention an impassioned counterattack against the prevailing view that the Jewish mother is at the root of the neuroses of her children. The article, "In Defense of the Jewish Mother" (12), is an impassioned defense of the nurturing, stimulating, and inspiring role of the Jewish mother—a description which in many ways fits Ros.

Because of their humanity, scientists have powerful drives. The Jewish sages taught us that without the *yetzer hara*—the evil instinct, or in Freudian terms, the id—the world's progress would come to a grinding halt. We would not procreate, nor would we conquer deserts or climb mountains. We would merely rock in our hammocks, sipping mint juleps under the trees. Those competitive instincts that are at the root of much misconduct in science are also the driving force that has made American biomedical research into the most successful scientific enterprise anywhere.

Yes, fraud is inevitable—in banks, in government, in business—and in the laboratory. As long as easy gains are to be had, and temptations are enticing, there will be frauds and dishonest researchers.

Is Scientific Fraud Increasing? Are frauds increasing? I have no data, and I believe that no reliable data exist. In fact, I am not at all sure that valid and meaningful data can be readily generated. Obviously only a small, but undefined, proportion of fraud is publicized. Clearly too, the volume of research and the number of scientists actually engaged in research has been increasing exponentially throughout this century. The involvement of the media and the accessibility of scientific work to the scrutiny of the lay public has likewise increased dramatically. All these factors operating simultaneously make it almost impossible even to guess whether the incidence of fraud is rising, falling, or stable. Those who are nostalgically inclined will suggest that things were better in the "good old days," but even nostalgia is not what it used to be. The debate over whether fraud is increasing is probably sterile and nonproductive. Fraud is here to stay, and we must contain it and attempt to uproot it.

The Fate of Whistleblowers. Perhaps even more serious than fraud itself are the attempts to deny it and cover it up. To me the most distressing aspect of the defensiveness of the scientific establishment is our attitude toward whistleblowers.

Our world of scientists, which proclaims to all that honesty and integrity are the cardinal rules of science, might be expected to reward, or at least to highly value, the courageous whistleblower who is protecting our sacred heritage, who is exposing those who erode the public's trust in science, those who pollute the literature—those who, in essence, disgrace us all. Yet repeatedly the whistleblower's popularity resembles that of Jeremiah, who was thrown into the pit for admonishing the populace and for bringing true, but pessimistic, tidings.

In September 1987, the American Association for the Advancement of Science sponsored a meeting on fraud and misconduct. Three scientists who exposed unequivocal misconduct in their surroundings were asked to describe their experiences. Each of them had been thoroughly vindicated and their charges found to be correct. Yet each was subjected to anguish, suffering, and hostility—none of which abated even when they were proven to be correct. Nor was the hostility confined to the colleagues they accused; it was generalized. Bruce Hollis (13), one of the three scientists who spoke at the meeting, concluded, "Although I am embarrassed to say this, I cannot recommend that junior scientists who discover scientific misconduct blow the whistle. That is, unless they want to experience immense personal suffering and a possible end to their scientific careers." Jerome Jacobstein (13), another one of the three who described their own experiences, concluded: "I am not optimistic."

Considering human nature, the response to exposers of fraud should not surprise us; hostility is clearly the rule, rather than the exception.

Perhaps the most dramatic example in our lifetime is the singularly monolithic refusal of the scientific establishment of post-World War II Germany to examine its history and the lessons of the Nazi medical war crimes. Almost total silence reigned for several decades after the war. A few years ago, when a distinguished German geneticist, Benno Müller-Hill, fascinated and disturbed by the Nazi war crimes and the role of the scientific establishment, devoted a sabbatical year to the subject and wrote *Deadly Science* (14), he was acclaimed by medical journals the world over. But in his home country he has been virtually ostracized.

But—lest we consider only others' problems—the second example strikes much closer to home. In the 1950s and 1960s, during the "golden" years of rapid flourishing of Western medical science, there was widespread abuse of patients. Informed consent was often not obtained, and patients were exposed to uncomfortable, often dangerous experiments without their knowledge or consent. This was the norm in most Western academic institutions. I know what I experienced during my residencies at Yale and Mount Sinai and some of the things I did personally, in violation of simple human decency—true, all for a good cause, but certainly not in any way ethical.

When Beecher, a distinguished professor of anesthesiology at Harvard, complained at a medical meeting in 1964 that "breaches of ethical conduct in experiments . . . are almost universal" (15), his good faith was attacked with vitriol. His shock at the attack led him to write his now classic article in *The New England Journal of Medicine* (16) describing 22 patently unethical studies which he culled from the medical literature.

In general Beecher fared much better than did Pappworth, a nonestablishment British physician, with his more dramatic and sensational and perhaps less precise book, *Human Guinea Pigs* (17). In the 1990 anniversary issue of the *British Medical Journal* (15), Pappworth described the enormous difficulties he encountered in getting his book published and the frigid rejections of it by the British medical establishment.

Self-Policing and Social Policing. We witness here a universal phenomenon—no one likes to be criticized, and it is a rare organization or institution that will police itself without external stimulation. George Bernard Shaw, in his biting comedy *Doctor's Dilemma,* most perceptively describes the impact of conflict of interest on the professional. Although the subject of his barbs is the surgeon, the same principles apply to the researcher and to the parent institution:

> That any sane nation, having observed that you could provide for the supply of bread by giving bakers a pecuniary interest in baking for you, should go on to give a surgeon a pecuniary interest in cutting off your leg, is enough to make one despair of political humanity. But that is precisely what we have done. And the more appalling the mutilation, the more the mutilator is paid. He who corrects the ingrowing toe-nail receives a few shillings: he who cuts your insides out receives hundreds of guineas, except when he does it to a poor person for practice.

If there are great rewards whose attainment can be accelerated by abuse of patients or by falsification of data, there will be individuals who will succumb. On the macro scale, to expect an institution to investigate vigorously a prominent researcher who brings it millions of dollars and enormous prestige goes against human nature and therefore does not work well.

David Baltimore, Nobel prizewinner, perhaps the most recent and one of the more notable causes célèbres, wrote a moving, if somewhat belated, acknowledgement of error (18): "I have come better to appreciate the legitimate role of government as the public sponsor of scientific research and to respect its duty to protect the public interest and hold the scientific community accountable for its stewardship of public funds." He goes on to acknowledge as well the critical need for a much more vigorous response by the scientific and academic community, and the need to protect whistleblowers.

His final sentence emphasizing the "importance of humility" may perhaps be the key to much of the sad episode. This closing sentence is a far cry from his statement less than two years earlier that "uninformed or malinformed outsiders cannot effectively review the progress of scientific activity" (18).

No individual, group, or institution is above external criticism. No group, to the best of my knowledge, no matter how well-motivated and talented, is capable of functioning at a sustained high level of intellectual and moral performance purely by self-motivation, considering itself above public scrutiny. Certainly, when the research establishment is almost totally dependent on public moneys and trust, it is being illogical and arrogant to maintain that it has the exclusive right of self-scrutiny.

Sadly but inevitably, we now have an Office of Scientific Integrity (OSI), with rules and regulations, standard operating procedures, and a self-sustaining bureaucracy. And we now have an Office of Scientific Integrity Review, established to check on the OSI. Human experimentation has also been similarly regulated. Until the National Institutes of Health began to insist on functioning committees on human experimentation at each institution, standards of researcher behavior were lax and variable. The scientific establishment can greatly enhance the effectiveness and utility of all these government agencies by cooperating fully in these activities rather than by adopting a passive-aggressive, stonewalling stance which will inevitably result in increasingly hostile and meddling interference with science and research.

For this presentation I reviewed much of the literature on the subject of research fraud. Many of the distinguished leaders of science have addressed the subject thoughtfully. Virtually without exception, they have sought factors within the organization of the scientific establishment and the way it operates to explain the phenomenon of misconduct.

Virtually every responsible, broadly based scientific and academic organization has now set up committees to examine the phenomenon of scientific misconduct and to produce guidelines on how to deal with it. These documents are, without exception, sound, thoughtful, and helpful. Individual institutions, such as Harvard University, have not only created detailed procedures for dealing with misconduct, but have attempted as well to institute preventive measures, such as a deemphasis on quantity of publication, and clear guidelines on conflict of interest. The profusion of conferences and meetings on the subject indicates the great interest in it. Certainly the later documents, which have the benefit of more experience and constructive criticism of earlier attempts, are more sophisticated and comprehensive than the first drafts.

Ultimate Social Causes. Yet few of the intrascientific discussions and documents have attempted to deal with the less proximate causes of misconduct and the broader philosophic problems. Petersdorf has touched briefly on the premorbid antecedents (19), citing the intense competitive situation during the premedical college years that creates an undesirable pattern of behavior. He also mentions the extraordinarily high incidence of admitted cheating on examinations by medical students and the widespread phenomenon of petty thievery of hospital drugs and supplies by house staff. But he does not venture much beyond this level.

I believe we are dealing with a problem involving not merely the organizational structure of research and science, but a much more pervasive and fundamental societal issue. The malaise and discontent surrounding the issue of fraud in science reflects an overall imbalance in the value systems of science and those of society as a whole.

Science and the Moral Sphere. Our century clearly can be characterized as one of unparalleled scientific and technologic advancement at a breathtaking and seemingly ever accelerating pace. Most of us have been active participants, not merely spectators, in this exhilarating experience. Human beings have attained powers and capabilities undreamt of even by science fiction writers of a few decades ago. The successes of science have had an understandably intoxicating effect on scientists themselves. Much of the public in its adulation of science had for a period of time even begun to share with the scientist the belief—or faith—that science is not only capable of solving the material problems of our universe but also holds the key to all significant human concerns. A common belief, still widely held, is that science itself can even provide the basis for a comprehensive theory of ethics, a science-based morality and code of human behavior.

Yet a more sobering analysis of history must suggest rather that the technological advances of this century have not been accompanied by parallel progress in the humanistic or moral sphere. Only incorrigible optimists can retain their blind faith in humanity in the shadow of Auschwitz, the gulags and Cambodia, and humanity's ongoing inhumanity in every corner of our planet. The naive, quixotic hope that behavior becomes progressively more civilized and ethical in parallel with scientific advances has been seriously discredited, if it was ever believable.

Most contemporary Western societies are pluralistic and have long abandoned unwavering allegiance to traditional sources of authority and guidance for standards of acceptable civilized and ethical behavior. The more thoughtful and introspective leaders of our societies have been seeking, at times groping, for substitutes to fill the vacuum left by the collapse of traditional sources of guidance. This process is an ongoing one in our Western societies but is far from resolved.

Many in the West are currently appropriately euphoric at the total collapse of the authoritarian and corrupt Soviet power which seemed so invincible not so long ago. Yet our joy at the downfall of dictatorship and the seeming triumph of freedom must not blind us to the serious moral crisis that still confronts not only these newly liberated societies but our own as well. The idealization of Western free competition that has justifiably so captivated the masses of Eastern Europe will inevitably be followed by deep disappointment if the adoption of this Western ideal is unaccompanied by a deeper message of social justice, to which the old regime at least paid lip service. The failures of Communism, as well as those of untrammeled capitalism, whose evils brought about the original Communist revolution, can legitimately be traced to moral failings. No society, of whatever economic or political ilk, can long survive if it is manifestly immoral and unjust. Fortunately, most humans seem to be endowed with some innate sense of justice and fairness.

The current preoccupation with the daily upheavals in the East must not divert our Western democracies from the ongoing and critical struggle to create a consensus with respect to the basic moral values in which our societies must be rooted—even if traditional bases have been shaken or even abandoned. Within the framework of fundamental values on which we should base our society, research and science represent not merely tools for material social progress but also important values to be cherished and nurtured. They deserve our respect and adulation for what they can accomplish to make our lives better. They also merit a vigorous and forceful defense against much of the anti-intellectualism rampant in the West, anti-intellectualism augmented by the growing forces of religious fundamentalism that see in science the devil reincarnated.

The Limitations of Science. But we must simultaneously be aware of the limitations of science, of the appropriate place of science in our hierarchy of values. Science is not all-powerful. It cannot by itself provide us with moral or ethical guidelines for society. It cannot even yield guidelines for its own self-regulation. Science and research are at the service of humanity and must not be permitted to dominate it. There are values higher than science and the pursuit of knowledge. Our tremendous curiosity, as positive a force as it is, must not be permitted to trample basic human rights, and society has an obligation to impose appropriate limits on science. As Albert Einstein said to a group of students: "Concern for man himself and his fate must always form the chief interest of all technical endeavours . . . in order that the creations of our minds shall be a blessing and not a curse to mankind. Never forget this in the midst of your diagrams and equations" (quoted in 7).

The hubris of scientists intoxicated with their impressive successes, if uncontrolled, can be harmful to society and, by bringing on an antiscientific and anti-intellectual backlash, may ultimately be autodestructive. One need not be particularly sensitive to feel the depreciation that science has suffered in recent years as a result of the various reports of fraud in science and of what has been perceived as the inadequate response of scientific leaders and institutions. I believe that more harm has been done by the somewhat arrogant response of some scientists than by the few cases of fraud in and of themselves. We are not above societal criticism and scrutiny, and we dare not project such an image.

The Primacy of Ethics. Maimonides, that great physician, scientist, philosopher, and rabbi, placed research in its proper niche. After praising investigation into nature and science and indicating that humans are obligated to indulge in such activities, he cautions (20) that one should not engage in "speculation" (scientific inquiry) until one has first "filled himself with the knowledge of what is forbidden and what is permitted" and likewise by all the commandments. In the modern vernacular his words mean that to avoid misuse of science, the development of moral standards and the cultivation of ethical behavior in each scientist must take precedence temporally, as well as in terms of priority, over scientific training. In the shadow of the egregious misuses of science we have experienced this century, and in light of the public attention focussed on unethical behavior by some scientists, this sage advice is more relevant than ever.

The German Jewish philosopher Hans Jonas said, "It is a question whether without restoring the category of the sacred, the category most thoroughly destroyed by the scientific enlightenment, we can have an ethics able to cope with the extreme powers which we possess today and constantly increase and are compelled to use" (21). To his question my reply would be an unequivocal "No."

References

1. Majerus PW. Fraud in medical research. J Clin Invest 1982; 70:213–217.
2. Donaldson VH. When things are not as they seem. J Lab Clin Med 1984; 103:491–496.
3. Koshland DE. Fraud in science. Science 1987; 235:141.
4. Kennedy TJ, Jr. Scientific fraud. J Med Educ 1988; 63:806–808.
5. Lock S. Misconduct in medical research: does it exist in Britain? Br Med J [Clin Res] 1988; 297:1531–1535.
6. Perspective: Fraud, irresponsible authorship and their causes. Ann Int Med 1986; 104:252–254.
7. Report: PNAS 1989; 86:9059–9074.
8. Science Scope. Science 1991; 251:507.
9. Glass B. Science and ethical values. Chapel Hill: University of North Carolina Press, 1967.
10. Piel G. The community of science. Address at Moscow State University, Nov. 25, 1985 (private comm.).
11. Merton RK. The sociology of science. Chicago: University of Chicago Press, 1973.
12. Blau ZS. In defense of the Jewish mother. Midstream, Feb. 1967, 42–49.
13. Hollis B, Jacobstein J. Whistle-blowing. The Scientist. Dec. 14, 1987:11–12.
14. Müller-Hill B. Tödliche Wissenschaft. Reimbek bei Hamburg: Rohwolt Taschenbuck, 1984.
15. Pappworth MH. "Human guinea pigs": a history. Br Med J [Clin Res] 1990; 301:1456–1460.
16. Beecher HK. Ethics and clinical research. N Engl J Med 1986; 274:1354–1360.
17. Pappworth MH. Human guinea pigs: experimentation on man. London: Routledge and Kegan Paul, 1967.
18. Baltimore D. Dr. Baltimore says "sorry." News and Views, Nature 1991; 351:94–95.
19. Petersdorf RG. The pathogenesis of fraud in medical science. Ann Int Med 1986; 104:252–254.
20. Ben-Maimon M. (Maimonides). Mishne-Torah, Hilkhoth Yesodei Hatorah. Sefer Haavodah 4, 13.
21. Jonas H. Quoted in Keyserlink, E.W. Sanctity of life or quality of life in the context of ethics, medicine and law. Ottawa: Law Reform Commission of Canada (130 Albert St., Ottawa K1A0L6), 1979.

Science and Social Policy

Enoch Gordis, M.D.

By the word "policy" I refer to enactment of regulations, laws, and other formal mechanisms whereby society exerts control over individual behavior. Interestingly, some of the decisions on policy involve ethical principles used in clinical practice (1, 2): autonomy, the right of persons to decide matters about their own future; distributive justice, the idea that everyone should be treated fairly, and that one group should not benefit at the expense of another; beneficence, the imperative to do good, and its converse, *primum non nocere* ("first do no harm"). Conflicts often arise between the principles of autonomy and distributive justice, especially in discussions of cost containment and rationing in health care. Here, interests of the individual may be sacrificed for what some perceive as a greater common good.

Three of the ways science interacts with policy are taken up below. Sometimes science has been decisive in providing evidence that led to policy creation. Sometimes science has created problems, but is capable with yet more effort of resolving them. Finally, on some important issues, decisions can be made only on the basis of nonscientific considerations.

Science Decisive in Establishing Policy

Minimal Legal Drinking Age. During the 1970s, there was a tendency for states to lower the minimum legal age for purchasing or possessing alcoholic beverages. Data showed clearly that highway accidents increased during this time, especially among young people. At the same time, laws varied from state to state, so that young persons were crossing state boundaries to obtain alcohol from jurisdictions with lower age limits. In 1984, therefore, Congress passed the Federal Uniform Drinking Age Act, which specified that federal highway funds would be withheld from any state that did not set 21 as the minimum legal age for purchase or public possession of all alcoholic beverages.

The state of South Dakota challenged the constitutionality of the law, which was eventually sustained by the Supreme Court (*South Dakota* v. *Dole,* 1987). As a result, 21 years is now the minimum legal "drinking" age throughout the United States. The law would not have been upheld without the *amicus* briefs filed by such constituency groups as the National Council on Alcoholism, Remove Intoxicated Drivers, and Mothers Against Drunk Driving. The decisive element in these briefs was scientific data on the epidemiology of highway deaths among the young. Thus, it was a combination of science and social advocacy that enabled the policy to be enacted.

Liver Transplants for Alcoholic Liver Disease. Liver transplants are expensive, and the conflict between autonomy and distributive justice as guiding principles is clear here. In considering health expenditures, some have thought that liver transplants of any sort should not be paid for from public funds, since the money might be better used, for example, for extensive prenatal care. But controversy has been even more extensive on liver transplantation for those with alcoholic liver disease. There has been, and still is, widespread bias against this practice. One reason is the persistent stigma associated with alcoholism: that is, the alcoholics "brought it on themselves," so they do not deserve another liver. Op-

---

Adapted from the author's Third Annual Marie Nyswander Memorial Lecture presented at Beth Israel Medical Center, New York City, May 11, 1989, edited and published as From science to social policy: an uncertain road. J Studies Alcohol 1991; 52(2):101–109, and the author's article Science and social policy problems: resolution of some, creation of others. Alcohol & Alcoholism 1991; Suppl. 1, 67–76.

From the Director, National Institute on Alcohol Abuse and Alcoholism, 5600 Fishers Lane, Room 16-105, Rockville, MD 20857. Address reprint requests to the author there.

ponents of liver transplantation for alcoholics have argued that recipients will all relapse into drinking, destroying their new liver, or would not comply with immunosuppressive therapy, resulting in rejection of the transplant. Even institutions willing to perform such transplants have sometimes required a period of abstinence before surgery so long that the patient would deteriorate to the point of inoperability.

However, convincing data emerged showing that the survival of alcoholic cirrhosis patients 1 year after liver transplantation and, in a subsequent study, 1½ years after transplantation, was no worse than for patients with any other kind of liver disease (3–5). These studies also showed that the rate of relapse to drinking was very low, and that alcoholic patients did comply with immunosuppressive therapy. As a result of this strong evidence, Medicare rules on liver transplantation list alcoholic liver disease as one of the conditions for which liver transplantation is reimbursable (56FR 15000-15018, April 12, 1991).

Science Resolves Issues It Has Created

Drinking and Driving. Many states have so-called *per se* laws that make it illegal for anyone to drive with a blood alcohol concentration above a specified level, regardless of whether an individual driver is demonstrably impaired at that level. These laws were developed because tests of impairment were chaotic, and because science had provided data on the average relation between blood alcohol level and impairment, and technology for measuring blood alcohol rapidly via the breath. But over the years, science has also demonstrated the validity of the concept of tolerance, that is, the adaptation of the nervous system to a drug as a result of chronic exposure to it. This means that experienced drinkers will show less impairment than novice drinkers on many tests. Some apprehended drinking drivers have claimed later in court that they were tolerant of alcohol and that the use of a *per se* level of blood alcohol to convict them was unfair.

Science might contribute to the resolution of this argument. People do not become tolerant to all effects of a drug at the same rate, and to some effects hardly at all. It is conceivable, however, that science will develop rapid, easily administered tests of impairment at the roadside which relate convincingly to essential driving skills. Were this to happen, we will have come full circle on the approach to drinking drivers. The *per se* laws could be dropped, and the conflict over them would become moot.

Objective Markers of Drinking. One of the policy issues arising from the discovery of the teratogenicity of alcohol ("fetal alcohol syndrome" and "fetal alcohol effects") is the question of establishing recommended limits for moderate drinking during pregnancy. That is, is there a threshold below which drinking is safe, or is alcohol in any quantity a danger to the fetus? The problem is that studies that have concluded that moderate drinking is dangerous are likely to include an unknown percentage of heavy drinkers who are underreporting their drinking.

Efforts are under way to develop biochemical measurements that could testify to the mean level of blood alcohol over a period of several weeks, even if the patient is abstinent at the time of sampling. Such a test would be analogous to the use of glycosylated hemoglobin for monitoring blood glucose in diabetes. The availability of objective markers of drinking would eliminate our dependence on self-report and provide a more rational basis for shaping policy on advice to pregnant women.

Policy Issues Science Cannot Resolve

Workplace Alcohol Testing. There has been a great deal of controversy in the United States over the intrusiveness of drug and alcohol testing as opposed to the public's right to safe transportation and the right of an employee not to be endangered by the drinking of a co-worker. Of all the drug tests that can be conducted in the workplace, alcohol testing is the most informative. Measurements of cocaine or marijuana in the urine give no indication of how long ago the drug was used or how the levels relate to impairment at the time the test is being done. Alcohol testing, however, quantifies the amount of alcohol actually present in the worker's body at the time of testing. Moreover, that level can be roughly correlated with the degree to which the worker may be impaired and at risk of causing damage.

At the same time, there is a history of nonpunitive efforts to treat alcoholics in the workplace through employee assistance programs. It has been suggested that routine or random alcohol testing in the workplace may jeopardize these programs by setting up an adversarial relationship between workers and employers, thereby discouraging alcoholics from seeking treatment. On the other hand, recent accidents such as the

Exxon Valdez disaster and a New York City subway crash suggest to many that, at least for sensitive positions, alcohol screening should be done. The science is available to implement an alcohol-testing policy, but the question of whether to implement it and for which category of workers cannot be resolved by science.

Genetic Testing for Employment and Insurance. It is possible today to test rapidly for genetic vulnerability to a number of diseases. This field has been named "predictive medicine" (6). Two primary policy worries in this field are the use of genetic testing as a condition for employment and its use as a condition for insurance.

During the last 15 years or so, impressive evidence has been assembled supporting the idea that a portion of the vulnerability to becoming alcoholic after exposure to alcohol is inherited (7). This evidence is of two sorts. From twin studies, it has been observed that the concordance for alcoholism in monozygotic twins is much higher than in dizygotic twins. In large adoption studies, it has been shown that infants born to a biological alcoholic parent and adopted out in infancy to a family without much drinking are far more likely to develop alcohol problems as adults than similar infants born to nonalcoholic biological parents and adopted out. We do not know the nature of the genetic vulnerability, how many genes are involved, or their chromosomal location. Furthermore, nongenetic influences account for much or even most of the variance in drinking. All these issues are now under intense investigation.

Genetic screening clearly has enormous potential for good. First, it provides information for counseling on all aspects of genetic conditions related to important decisions in life—whether to marry, what career to choose, whether to have children. Second, it might help identify candidates for targeted prevention effort; this is one of the goals of alcoholism genetics research. Of course, in the case of alcoholism, genetic screening would predict only risk, not disease presence. A strong environmental influence is necessary, here an exposure to an exogenous agent, alcohol.

In genetic screening for insurance purposes, another benefit would be the fair distribution of the cost of insurance. Allegedly, if high-risk people could be identified, they would be charged a higher premium than everybody else, spreading the risk more equitably. In the case of employment testing, screening for genetic vulnerability to toxic effects of exogenous substances might reduce the risks of exposure to hazardous materials in the workplace.

The insurance industry is becoming seriously interested in the implications of genetic testing. According to Dr. Robert Pokorski of the American Council of Life Insurance (quoted in 8), "The whole foundation of insurance is based on the fact that we and the insurance applicant are operating with equal levels of knowledge and ignorance. If they know something, we want to know it. If they don't know something, we don't need to know it."

Obviously, general genetics testing will be of great public interest and concern for some time to come. It presents serious problems as well as benefits. The first is the issue of stigmatization, the possibility of labeling somebody as different and defective, possibly less acceptable. A second problem is the question of intrusion into some of the most private aspects of our being. A third is the possibility that the genetic determination of occupational hazard risk will give employers an excuse to discriminate against whole classes of people when they apply for jobs. And finally, everyone is at risk for something, and how the insurance rates will change is a matter of concern.

Science and technology may soon be able to provide genetic screening, but values outside science will play the decisive role in determining whether genetics testing becomes accepted public policy.

Conclusion

Scientists have two roles: one is to do good science, the other is to be a spokesperson for science in the creation of rational social policy. In the many instances in which science alone cannot decide an issue, we can do no better than to learn from Ros and Sol how mind and heart should operate together in matters of great human concern.

References

1. Levine RJ. Ethics and regulation of clinical research. Baltimore, MD: Urban & Schwarzenberg, 1986.
2. Young E. Alpha and omega: ethics at the frontiers of life and death. Boston: Addison-Wesley, 1989.
3. Starzl TE, Demetris AJ, Van Thiel D. Liver transplantation. N Engl J Med 1989; 321:1014–1022.
4. Starzl TE, Demetris AJ, Van Thiel D. Liver transplantation. N Engl J Med 1989; 321:1092–1099.
5. Starzl TE, Van Thiel D, Tzakis AG, et al. Orthotopic liver transplantation for alcoholic cirrhosis. JAMA 1988; 260:2542–2544.
6. Stone DA. Predictive medicine: implications and effects. Compensation & Benefits Manage 1990; 6:157–162.
7. Devor EJ, Cloninger R. Genetics of alcoholism. Ann Rev Genet 1989; 23:19–36.
8. Berlfein J. Genetics testing: health care trap. Los Angeles Times, April 30, 1990, p. B2.

Neonatal Hypothyroidism in India

N. Kochupillai, M.D.

Abstract

Nutritional iodine deficiency and endemic goiter are widely prevalent in developing countries, affecting an estimated 1 billion people. Recent studies in India, using radioimmunoassay methods, have shown that the incidence of neonatal hypothyroidism in endemic goiter regions is more than a hundredfold higher than the incidence in nonendemic regions. This knowledge has given a new momentum to the iodine prophylactic program in the country, resulting in the protection of hundreds of thousands of newborns from mental retardation.

Diversity in the clinical epidemiology of any given disease is well recognized. Such diversities often parallel variations in ecology and socioeconomic characteristics as well as ethnic and cultural traits of the affected populations. Several clinical disorders of the thyroid, as seen in developing countries like India, are good examples.

Environmental iodine deficiency (EID), which causes endemic goiter (1), is among the most widely prevalent of these ecological factors. Until recently, it was assumed that the adaptive response of the human thyroid to EID-related nutritional iodine deficiency (NID) (2) resulted in euthyroidism in the vast majority of affected subjects. The absence of clinical features of overt hypothyroidism among endemic populations was a major reason. Thus NID and endemic goiter were given very low priority in the illness-prevention programs of developing countries. A striking example, as recent as 1976, is in the monograph "Nutrition in Preventive Medicine" published by the World Health Organization (3); in the introductory chapter the authors, while rightly acknowledging the wide prevalence of EID and endemic goiter, attribute relatively low significance to it as a health problem. Unsurprisingly, NID and endemic goiter continue to affect hundreds of millions of people in the developing world, even though simple and effective methods of eradication are available.

Nutritional Iodine Deficiency and Endemic Goiter in Developing Countries

NID is among the most widespread of all human malnutritional diseases. According to a recent estimate, about a billion people living in the developing countries of Asia, Africa, and Latin America are at risk; a quarter of them are goitrous (4) (Table 1).

About 28% of the population at risk live in the SE Asian region. Here, a variety of ecological geoclimatic, sociocultural, and economic factors make NID and related health problems particularly severe and widespread (5, 6). In India alone, it is estimated that 150 million people are at risk and approximately 40 million are goitrous (8). Thirty years ago the etiology of endemic goiter in India was shown to be due to NID (7). A national goiter control program using iodized salt was launched soon thereafter. However, a review of the program after two decades revealed a total failure in implementation (8).

Similar reports of failure to implement programs of iodine prophylaxis are well-known in almost all countries of the region. A major reason for this failure was the prevailing perception that

---

Fund support for this work was provided by the Ministry of Health, Government of India.

From the Department of Endocrinology, Metabolism & Diabetes, R. No. 3091, All India Institute of Medical Sciences, New Delhi 110029 India. Address reprint requests to the author, who is Associate Prof. of Medicine and Head of Dept. of Endocrinology, Metabolism & Diabetes, at that address.

NID results in a largely "cosmetic" problem of goiter and hence was given only low priority among the many pressing health problems that demand fund allocation.

Functional Thyroid Status in Goiter Endemias of India

Clinically overt hypothyroidism, like cretinism, is relatively rare in most endemic regions of India and other developing countries. Early attempts to measure thyroid hormones in endemic goitrous subjects, using nonspecific techniques such as protein-bound iodine, showed relatively low values (7). However, not much significance could be attached to this finding, for a variety of technical reasons, particularly in the absence of supporting clinical evidence.

However, with the development of radioimmunoassay (RIA) (9), methods soon became available for specific quantitation of thyroid-related hormones such as T4, T3, and TSH. In 1973, with technical help from the laboratory of Prof. R. Hall of the University of Newcastle upon Tyne in the U.K., we carried out a limited study to sensitively and specifically assess the thyroid status of endemic goitrous subjects in the state of Uttar Pradesh in the northern Indian Gangetic Plains. These studies revealed the following striking facts on functional status of the thyroid in an endemic-goiter region (10):

- >50% of the goitrous subjects showed elevated basal TSH level and augmented TSH response to TRH, indicating subclinical hypothyroidism
- There was an inverse relationship between goiter size and T4 levels and peak TSH response to TRH
- Though relatively high levels of circulating T3 could be demonstrated in goitrous subjects, there was no correlation between circulating T3 levels and TSH response to TRH.

These observations demonstrated for the first time that a majority of goitrous subjects in an endemic region may be functionally decompensated and hypothyroid, although no clinically overt hypothyroidism was evident. Subsequently, several studies in other endemic-goiter areas have confirmed these findings (11–14).

Neonatal Thyroid Status in Endemic-Goiter Regions of India

The demonstration of high prevalence of thyroid failure among adult goitrous subjects raised the question of the extent of neonatal hypothyroidism. In 1975 I was awarded the Solomon A. Berson Research Fellowship to work with Dr. Yalow. I later learned that it was the perspicacity of Dr. Yalow that led to this award. Having read our *Lancet* paper (10), she foresaw the potential importance of wide application of RIA techniques to investigation of the significance of nutritional iodine deficiency as a health problem in developing countries. When I joined the Berson Research Laboratory in 1975, Dr. Yalow encouraged me to develop cost-effective techniques to measure thyroid hormones, with a view to enabling me to return to India with techniques to study the clinical thyroid problems in the endemic-goiter region.

TABLE 1

Global Nutritional Iodine Deficiency and Iodine-Deficiency Disorders

Region	Population at risk (millions)	Goiter (millions)
Africa	227	39
Latin America	60	30
SE Asia	280	100
China and other Asia	400	30
Eastern Mediterranean	33	12
Total	1000	211

Adapted from WHO draft report for World Health Assembly, May 1990 (ref. 4).

Development of Strategies for Screening. On returning to India I established a Radioimmunoassay Laboratory in the Department of Medicine, All India Institute of Medical Sciences, with funding support from the Department of Science and Technology, Government of India. The RIA procedures developed in the Berson Laboratory (15) were established, and new procedures were developed and adaptively used in this laboratory. These technical accomplishments and field strategies ultimately enabled us to successfully organize a cost-effective neonatal hypothyroid screening program in the socioeconomically backward endemic regions of India (16–18).

Radioimmunoassay Techniques for Screening. Low cost and high assay sensitivity were two important requirements to successfully organize neonatal hypothyroid screening in India. The technique developed in the Berson Laboratory for preparing and purifying high specific activity radioactive T4 tracer was of great help. The high-energy T4 antiserum, required for measuring picogram quantities of T4 from dried filter paper blood spots, was prepared by immunizing thyroidectomized rabbits (made euthyroid by T3 therapy) with bovine thyroglobulin.

TABLE 2

Incidence of Neonatal Hypothyroidism in Endemic-Goiter and Nonendemic-Goiter Regions of India

Region/ district	Goiter prevalence (%)	Urinary iodide excretion (Follis group)	Incidence of neonatal hypothyroidism (per 1000 birth)
Deoria UP	80	V*	133
Gorakhpur UP	70	V*	85
Gonda UP	60	V*	75
Delhi	29	II†	6
Coastal Kerala	1.3	NA	1

NA, not available; UP, Uttar Pradesh.

* Follis group V: >50% excrete <25 μg of urinary iodine per g of creatinine.

† Follis group II: none excrete <25 μg of urinary iodine per g of creatinine.

A specific and sensitive RIA for thyroxine was developed and validated using the T4 tracer and T4 antibody prepared in our laboratory. This assay was then adapted for the dried blood spot assay essentially as described by Larsen and Broskin (19). Cord blood rather than heel-prick blood was used in our screening program because it was impossible to get blood by the heel prick method from newborns in the endemic regions. From every blood spot in which the measured T4 was below 3 μg% (2SD below normal mean T4), additional spots were assayed for TSH, using reagents obtained from the National Institutes of Health in Bethesda and adopting techniques described by Larsen et al. (20). If the TSH measured in such spots was more than 50 μU/mL, a diagnosis of neonatal hypothyroidism was made.

The cost of screening a neonate using this technique was estimated to be less than 50 U.S. cents per child screened (18).

Field Strategies. Many logistical problems at the field level had to be overcome to successfully organize a screening program for neonatal hypothyroidism in remote endemic districts of India. The major problems we encountered included difficulties in obtaining blood samples from newborns, poor coverage of birth by hospitals, and lack of motivation and cooperation among lay people and health personnel. We overcame these problems by adopting appropriate measures to motivate district residents and health personnel to cooperate in the program. We simplified procedures required to send blood spots to the laboratory at Delhi, by adopting a cord-blood spot based on randomized screening and by distributing postage-paid addressed envelopes with filter paper strips among health workers and even expectant mothers (17).

Neonatal Hypothyroidism and Brain Damage. Adopting the techniques and field strategies outlined, we screened newborns from three regions of India: (a) three severely iodine-deficient, endemic districts of the Gangetic Plains of Uttar Pradesh; (b) the hospitals of the capital city of Delhi, where a mild degree of environmental iodine deficiency and endemic goiter are prevalent (22); and (c) two coastal districts of the southern state of Kerala, where neither the environmental deficiency nor goiter are prevalent. Over 20,000 newborns were studied from these three regions during the three-year period 1982–84.

The results (Table 2) showed an incidence of neonatal hypothyroidism in the range of 75–133 per 1000 births in the three endemic districts, much higher than that in Delhi hospitals and in coastal Kerala. Subsequent to the discovery of this high incidence, we did field studies to assess two important parameters of brain development (IQ evaluation and audiometric studies for occurrence of sensory-neural deafness) known to be affected by fetal/neonatal hypothyroidism in populations with a high incidence of neonatal hypothyroidism. The results showed a much higher gross shift to the left of the distribution of IQ scores among primary school children from endemic-goiter villages than in children from nonendemic control villages (21, 23). The audiometric testing revealed sensory-neural hearing loss in one of every 5 subjects screened from the endemic-goiter villages (23).

The results of our studies, summarized, permit the following conclusions:

- The incidence of neonatal hypothyroidism, as reflected in cord-blood T4 and TSH levels, is more than a hundredfold higher than that observed in nonendemic regions of India or reported from developed countries of the West.
- The incidence varies from 0.6% to 13%, depending on the severity of iodine deficiency as assessed by the pattern of urinary iodine excretion in the affected population.
- In areas with high incidence and iodine deficiency, a significant proportion of the population may have varying degrees of mental subnormality and sensory-neural hearing loss.

Impact on Nutritional Iodine Deficiency in India

By 1983 our publications on high incidence of neonatal hypothyroidism and related brain damage risk in endemic areas inhabited by millions of people in India began attracting media and public attention. A large number of newspaper stories and parliamentary questions directed government attention to the problem of NID and eventually we were able to communicate personally, through a video presentation, to the then prime minister, Mrs. Indira Gandhi, the newly discovered sinister aspects of NID as a health problem.

These developments led to a real change in the attitude of health planners and policy makers to the nutritional deficiency as a health problem in India. The federal funding allocations for iodine prophylaxis were increased tenfold during the 7th plan period, and a national goiter prevention program was included in the prime minister's 20-point program of priority. By 1985–86 a national policy for universal iodation of salt in India was approved by the Central Council for Health and Family Welfare. This policy is to be implemented nationwide by the end of 1992.

Impact of Salt Iodation on Neonatal Hypothyroidism

In view of the serious nature of the nutritional deficiency and its health consequences in the state of Uttar Pradesh, as demonstrated by our studies, the government of India initiated emergency measures to implement iodized salt prophylaxis there. As a result, by October 1987, a policy of universal iodation of edible salt was adopted for the state of Uttar Pradesh, which has an estimated population of 110 million.

Our Department was to monitor the effective implementation of the program statewide as well as to evaluate the impact of the program on the incidence of neonatal hypothyroidism. Accordingly, we developed a three-pronged system for monitoring and evaluating the ongoing iodine prophylactic program. The components of this program are:

- assessment of the level of iodation of edible salt achieved at the consumer level by analyzing a randomized and statistically valid number of salt samples collected from retail outlets all over the state
- evaluating the actual status of iodine nutrition by assessing the pattern of urinary iodine excretion in the postiodation phase
- reevaluating the incidence of neonatal hypothyroidism in the same 3 districts in the postiodation phase

The results of this ongoing monitoring and evaluation (24) tell a remarkable story: 95% or more of salt samples collected from retail outlets were found to be iodized, the majority at a level of iodation satisfactory for ensuring normal iodine uptake. Whereas in the endemic districts the preiodation urinary iodine excretion pattern indicated extreme iodine deficiency in more than 50% of subjects, after iodation none of the people screened excreted less than 100 μg of iodine per g of creatinine. The incidence of neonatal hypothyroidism declined impressively with iodation in all 3 endemic districts (Table 3).

TABLE 3

Incidence of Neonatal Hypothyroidism in Three Endemic-Goiter Districts of Uttar Pradesh State, India, Before and After Successful Salt Iodation

District	Incidence preiodation	Per 1000 birth postiodation*
Deoria	133	16
Gonda	75	9
Gorakhpur	85	17

* Data on the basis of 5500 newborns in the 3 districts screened so far; studies are ongoing.

The Indian experience in tackling the health problem of nutritional iodine deficiency is unique. It illustrates how new knowledge about several sinister aspects of an age-old and neglected health problem was gained by wide and cost-effective application of radioimmunoassay in remote endemic-goiter regions of a developing country. It also illustrates how such new knowledge can help generate the required social awareness and political will for eradication of the problem. The ongoing successful effort to eradicate nutritional iodine deficiency from India by 1992 is saving hundreds of thousands of newborns from the scourge of mental retardation. It gives me great pleasure to acknowledge the creative role played by Dr. Rosalyn S. Yalow in this happy development.

References

1. Beckers C, Delange F. Iodine deficiency: etiology of endemic goiter. In: Stanbury JB, Herzel BS, eds. Endemic goiter and endemic cretinism. New York: John Wiley & Sons, 1980:199–217.
2. Stanbury JB, Brownell GL, Riggs DS, Perinetti H, Itoiz J, Del Castillo EB. Endemic goiter: the adaptation of man to iodine deficiency. Cambridge: Harvard University Press, 1954:1–209.
3. Beaton GH, Bengoa JM. Nutrition of health in perspective: an introduction. In: Beaton GH, Bengoa JM, eds. Nutrition in preventive medicine. Geneva: World Health Organization, 1976:13–20.
4. World Health Organization Draft Report to the World Health Assembly, Geneva, May 1990.
5. Kochupillai N. Impact of iodine deficiency on human resources development. In: Chandra R, ed. Progress in

food and nutrition sciences, 13. New York: Pergamon Press, 1989:1–15.

6. Kochupillai N, Ramalingaswami V, Stanbury JB. Present status of endemic goiter as a problem of public health in South East Asia. In: Stanbury JB, Hetzel BS, eds. Endemic goiter and endemic cretinism. New York: John Wiley & Sons, 1980:101–122.
7. Ramalingaswami V, Subramanian TAV, Deo MG. The etiology of Himalayan endemic goiter. Lancet 1961; I:291.
8. Pandav CS, Kochupillai N. Endemic goiter in India: prevalence, etiology, attendant disability, and control measures. Indian J Pediatr 1982; 50:1259–1271.
9. Yalow RS, Berson SA. Assay of plasma insulin in human subjects by immunological methods. Nature 1959; 184:1648–1649.
10. Kochupillai N, Karmarker MG, Weightman D, et al. Pituitary-thyroid axis in Himalayan endemic goiter. Lancet 1973; I:1021–1024.
11. Maberly GF, Eastman CJ, Corcoran JM. Thyroid hormone response to thyrotropin releasing hormone stimulation in subjects from endemic goiter regions of Sarawak, Malaysia. Aust News J Med 1979; 9:385–390.
12. Bourdoux P, Thilly C, Delange F, Ermans HM. A new look at old concepts of laboratory evaluation of endemic goiter. In: Dunn JT, Pretell EA, Daza CH, Viteri FE, eds. Towards the eradication of endemic goiter, cretinism, and iodine deficiency. Washington, DC: Pan American Sanitary Bureau, Regional Office of the World Health Organization, 1986; PAHO scientific publication no. 502:115–129.
13. Bachtarzi H, Benmiloud M. TSH regulation and goitrogenesis in severe iodine deficiency. Acta Endocrinol (Copenh) 1983; 103:21–27.
14. Chopra IJ, Hershman JM, Hornabrook RW. Serum thyroid hormone and thyrotropin levels in subjects from endemic goiter regions of New Guinea. J Clin Endocrinol Metab 1975; 40:326–333.
15. Kochupillai N, Yalow RS. Preparation, purification and stability of high specific activity ^{125}I-thyronines. Endocrinology 1978; 102:128–135.
16. Kochupillai N, Godbole MM, Pandav CS, Karmarkar MG, Ahnja MMS. Neonatal thyroid status in iodine deficient environment of the sub-Himalayan region. Indian J Med Res 1984; 80:293–299.
17. Kochupillai N, Pandav CS. Neonatal chemical hypothyroidism in iodine deficient environments. In: Hetzel BS, Stanbury JB, eds. Prevention and control of iodine deficiency disorders. New York: Elsevier Science Publishers, 1987:85–93.
18. Kochupillai N, Jayasuryan N, Godbole MM, Pandav CS. Benefit and cost of application of radioimmunoassay in tuberculosis and iodine deficiency disorders: two major health problems of developing countries. In: Albertini A, Ekins RP, Galen RS, eds. Cost/benefit and predictive value of radioimmunoassay. New York: Elsevier Science, 1984:203–212.
19. Larsen PR, Broskin K. Thyroxine immunoassay using filterpaper blood samples for screening neonates for hypothyroidism. Pediatr Res 1976; 96:107.
20. Larsen PR, Merker A, Parlow AF. Immunoassay of human thyroid stimulating hormone using dried blood samples. J Clin Endocrinol Metab 1976; 42:987.
21. Mehta M, Pandav CS, Kochupillai N. Intellectual assessment of school children from severely iodine deficient villages. Indian Pedriatr 1987; 24:467–473.
22. Pandav CS, Kochupillai N. Endemic goiter in Delhi. Indian J Med Res 1980; 72:81–88.
23. Kochupillai N, Pandav CS, Godbole MM, Mehta M, Ahnja MMS. Iodine deficiency and neonatal hypothyroidism. Bull WHO 1986; 64(4):542–551.
24. Kochupillai N. Reports to the Ministry of Health and Planning Commission, Government of India on Monitoring and Evaluation of Salt Iodation in the State of Uttar Pradesh, India, 1989–90.

Rosalyn S. Yalow delivering the Nobel Lecture.

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society

Regulation of Gastrointestinal Functions

Physiology and Pathophysiology of Gastrin

John H. Walsh, M.D.

This personal chronicle of my research in the gastrin field (1, 2) is not intended to be a comprehensive review of the field and ignores excellent contributions by numerous other researchers.

Metabolism of Molecular Forms of Gastrin. When pure preparations of big gastrin (G34) became available from Gregory and Tracy, comparison of the metabolic clearance and biological activity of this form with little gastrin (G17) actions had high priority. It was found that the clearance rate of G34 was approximately 6 times slower than clearance of G17 in dogs and in humans. The clearance of minigastrin (G14) was similar to the clearance of G17.

Organs responsible for gastrin removal from the circulation were studied in anesthetized dogs by measuring arteriovenous differences in gastrin concentrations. The concentration of gastrin in jugular, femoral, renal, and mesenteric veins was 20%–30% lower than arterial concentrations, suggesting that mechanisms for removal of circulating G17 are widely distributed in the body and not restricted to the kidney. The liver does not appear to play a significant role in gastrin clearance.

The biochemical pathways of gastrin degradation have been studied by determining enzymatic activities in tissue extracts that act upon gastrin. An amidase activity was identified in liver that converts N-acylated pentagastrin to its free acid, but this pathway probably is not of major importance in clearance of larger forms of gastrin. A more likely catabolic pathway involves neutral endopeptidase EC 3.4.21.11 (enkephalinase), a membrane-bound peptidase that has especially high affinity for substance P and GRP. This enzyme was identified and partially purified from pig stomach (3), and is known to be widely distributed throughout the body. Metabolism of gastrin and cholecystokinin by purified pig stomach endopeptidase 24.11 led to removal of the carboxyl terminal phenylalanine amide residues that are essential for biological activity (4). The affinity of this enzyme for gastrin is lower than for some neuropeptides, so other mechanisms may exist that are more efficient for gastrin degradation.

Acid-Stimulating Activity of Molecular Forms. Both sulfated and nonsulfated forms of G17 and of G14 had similar acid-stimulating potency. The biological activity of G34 initially was found to be less than that of G17. This was somewhat surprising because Gregory had reported that acute intravenous injections of G34 produced both prolonged and accentuated acid responses. The infusion studies done in our laboratory utilized purified natural G34 that may have been exposed to oxidizing conditions during rationing of the rare material.

We subsequently obtained synthetic human G34 and G17 from Professor Wünsch and repeated the comparisons of clearance and potency in humans. Although the clearance rates of G34 and of G17 were nearly identical to those found in the earlier study, circulating G34 was approximately equal to G17 in acid-stimulating potency. These latter results strongly suggest that there is little difference in acid-stimulating activity of G17 and G34 and that radioimmunoassay measurements with antibodies that react similarly with all C-terminal amidated forms of gastrin

---

From the Department of Medicine, Division of Gastroenterology, UCLA School of Medicine, Los Angeles, CA. Address reprint requests to the author, Dorothy and Leonard Straus Professor of Medicine, Chief, Division of Gastroenterology, UCLA School of Medicine, 44-146 Center for the Health Sciences, 10833 Le Conte Avenue, Los Angeles, CA 90024-1684.

probably reflect acid-stimulating activity of circulating gastrin. Oxidized gastrin probably does not occur in vivo. The only reliable method of identifying this biologically inactivating chemical change in vitro is by HPLC or some similarly powerful analytical separation technique. Further comparison of synthetic human G34 and G17 indicated that the two peptides had similar potency for stimulating lower esophageal sphincter contraction in humans.

Gastrin Release by Food and Food Components. A mixed meal was found to release gastrin, and atropine prolonged the duration of response to the meal without altering the initial response. In a patient with marked basal hypergastrinemia due to pernicious anemia, atropine produced no change in plasma gastrin. This study showed that food caused phasic release of gastrin and that prolonged achlorhydria caused tonic release of gastrin through noncholinergic mechanisms.

The components of a meal that caused gastrin release were studied. Amino acids were potent stimulants, whereas glucose and fat were only weak stimulants. Undigested albumin did not cause significant stimulation of gastrin release. The amount of gastrin released and acid secretory responses were positively correlated. A direct comparison of individual amino acids revealed that phenylalanine and tryptophan were the most potent stimulants of both gastrin release and acid secretion and that several amino acids produced no stimulation. Similar results were obtained in dogs.

Inhibition of Gastrin Release at Low Intragastric pH. Acidity of gastric contents affects gastrin responses to meals. The gastrin and acid responses to a mixed amino acid meal were examined while intragastric pH was maintained at 5.5, 4.0, 3.0, and 2.5 in normal subjects and in patients with duodenal ulcer disease. The critical pH range for inhibition of gastrin responses to amino acids was found to lie between 2.5 and 3.0. The inhibition at low pH was found to be more marked in control subjects than in duodenal ulcer patients.

Gastrin Release by Antacids, Beer, and Wine. Calcium carbonate was known to cause "rebound" gastric acid hypersecretion when used as an antacid for treatment of peptic ulcer disease. Single oral doses of calcium carbonate stimulated gastrin release, but sodium bicarbonate did not. Subsequently it was found that continuous neutralization of gastric contents with sodium bicarbonate began to cause an increase in serum gastrin after five hours. Aluminum-containing antacids cause gastrin release, but the effect is blocked by cimetidine, implying that cation ionization by acid is involved in the stimulation. Gastrin also is released by chemical substances other than ethanol that are continued in beer and wine but not in whiskey.

Release of Gastrin from Duodenum. Evidence for release of duodenal gastrin was obtained by comparing serum gastrin responses to food in patients with prior antrectomy who had either Billroth I gastroduodenostomy or Billroth II gastrojejunostomy. When food came into contact with the duodenum, gastrin responses during the second postprandial hour were similar to those found in unoperated-on duodenal ulcer patients, although the first-hour response was blunted. In contrast, the Billroth II patients had very little gastrin response to food. In patients with vagotomy and no antral resection, basal gastrin and postprandial responses were approximately twice normal. Yalow and Berson had found previously that the big gastrin and little gastrin were present in almost equal concentrations in duodenum, whereas little gastrin represented more than 90% of antral gastrin. We showed that big gastrin (G34) was preferentially released into the blood in patients with antral resection and Billroth I type gastroduodenostomy.

Vagal and Cholinergic Regulation. Cholinergic inhibition of gastrin release was demonstrated in humans by the finding that anticholinergic agents enhance gastrin responses to neural stimulation when intragastric pH is maintained at a constant level. Atropine accentuated the gastrin response to insulin hypoglycemia in ulcer and nonulcer subjects when gastric pH was maintained at 5.5. In dogs, gastrin responses to insulin could be inhibited by atropine, but only at extremely high doses.

Gastric distention with mannitol solution was produced by use of a barostat to maintain constant intragastric pressure increases of 5, 10, and 15 cm water for one hour. Acid secretion was stimulated most by a 5-cm pressure increase, but serum gastrin was not changed over baseline, implying that the stimulation of acid was mediated by a reflex that did not involve gastrin release.

Cephalic-vagal stimulation by sham feeding increased acid secretion out of proportion to the small increases in serum gastrin that were obtained. Cephalic stimulation also enhanced the acid secretory responses to intragastric meals of saline, glucose, and mixed food. Low doses of atropine increased basal gastrin concentrations when the stomach was perfused with pH 5.0 sodium chloride solution, and atropine enhanced the serum gastrin response to sham feeding. Thus, cholinergic inhibitory mechanisms that were independent of gastric pH acted to limit the gastrin responses to distention and to cephalic-vagal stimulation.

It was found that gastrin release by sham feeding was abolished when the intragastric pH was lowered to 2.5 for 75 min prior to sham feeding, and that the acid response was diminished. Atropine reversed the inhibition of gastrin release at pH 2.5. After atropine, gastrin responses to sham feeding were enhanced approximately threefold over the response obtained with intragastric pH at 5.0 in the absence of atropine, and gastrin responses were almost identical regardless of intragastric pH. In another study, atropine at a dose of 5 μg/kg significantly enhanced the gastrin response to sham feeding in normal subjects, but a higher dose of 15 μg/kg caused significant suppression. These observations could be explained if acid releases a local inhibitor of vagally stimulated acid secretion, such as somatostatin, and a low dose of atropine prevents the release of this inhibitor.

Acute distention of the stomach with 700 mL volumes of isotonic saline caused rapid increases in serum gastrin that peaked at 7.5 min and were enhanced more than twofold by prior treatment with low-dose atropine. Similar results were obtained when the distending solution was given at pH 2.5 or at pH 5.0. The failure to observe an effect of low pH with distention contrasts to the marked inhibition of sham feeding gastrin responses found with gastric acidification. This difference could be explained by a time requirement for low pH to produce inhibition, since the sham feeding studies were preceded by intragastric acidification for 75 min, whereas the distention studies were performed immediately after gastric aspiration.

Distention-induced gastrin release appears to be mediated by a β-adrenergic mechanism. The gastrin response to acute distention in humans was blocked by propranolol, and the exaggerated response usually obtained after atropine pretreatment was markedly inhibited by propranolol. The α-adrenergic antagonist phentolamine had no effect.

The enhanced gastrin response to meals produced by an anticholinergic agent was probably due to alteration in intragastric pH. When gastric pH was maintained at 5.0 by intragastric titration, atropine did not cause a significant increase in the gastrin response to a steak meal. In fact, very low doses of atropine caused modest inhibition of the gastrin response to amino acid meals in humans, whereas higher doses had no effect. These results indicate important differences in the mechanisms of gastrin release stimulated by food versus gastrin release stimulated by neural activation.

Effects of Vagal Denervation of Stomach. Vagotomy has complex effects on gastrin release and gastric acid secretion. In humans, proximal gastric vagotomy causes hypergastrinemia and exaggerated gastrin responses to meals. Sensitivity to gastrin and maximal acid responses to gastrin are both decreased after vagotomy. Subjects whose parietal cells are least sensitive to gastrin tend to have the highest basal and postprandial serum gastrin concentrations. Vagotomy does not alter gastrin clearance rates. It is likely that hypergastrinemia in some patients with diabetes mellitus is due to autonomic neuropathy that simulates the effects of vagotomy on the stomach.

In dogs vagotomy leads to basal hypergastrinemia that appears within 24 h after the procedure, too early to be due to gastrin cell hyperplasia and consistent with removal of a tonic vagal inhibitor of gastrin release. The earliest response to vagotomy, however, is a decrease in plasma gastrin during the first two hours after the procedure (5). The vagal inhibitory factor appears to involve the nonantral stomach because antral denervation does not lead to an increase in serum gastrin concentrations.

Selective vagal denervation of the acid-secreting portion of the stomach by proximal gastric vagotomy abolishes the acid secretory response to sham feeding and decreases the acid response to food by about 60%. When gastric pH was maintained at 5.0, gastrin responses to food were exaggerated in vagotomized patients compared with unoperated-on control patients. This enhanced gastrin release could reflect loss of vagal inhibitory mechanisms located in the proximal stomach or could be a result of antral gastrin cell hyperplasia.

A small but significant gastrin response to sham feeding was obtained after proximal gastric vagotomy, indicating that vagal fibers to the antrum were intact but that the cholinergic inhibitory mechanism that is antagonized by atropine during sham feeding in the intact stomach is not removed by proximal vagotomy. Antral vagotomy in dogs does prevent gastrin release by sham feeding. Intravenous infusion of the muscarinic cholinergic agonist bethanechol in subjects with prior proximal gastric vagotomy failed to inhibit basal gastrin release. An experiment that should be done, but apparently has not yet been undertaken, is to determine the effect of low doses of atropine on gastrin responses to sham feeding in vagotomized subjects.

Stimulatory reflexes that lead to the release of antral gastrin in dogs can be initiated by distention of vagally innervated oxyntic pouches (Pavlov pouches) when antral pouches also are present and maintained at neutral pH.

Gastrin Release by Bombesin and Gastrin-Releasing Peptide. Bombesin is a peptide obtained from the skin of a frog, *Bombina bombina,* that causes potent stimulation of gastrin release and of gastric acid secretion. The stimulation of acid secretion is secondary to gastrin release and can be prevented by resection of the gastric antrum or by administration of gastrin antibody (6). Very low doses of intravenous bombesin caused stimulation of gastrin release and parallel stimulation of acid secretion in humans. Bombesin also is a potent inhibitor of gastric emptying in humans, but this effect does not appear to be related to gastrin release.

The role of gastrin-releasing peptide, the endogenous form of mammalian bombesin, in regulation of gastrin release is not clear. It could contribute to neurally mediated release of gastrin but does not appear to play any role in food-stimulated gastrin release. Bombesin-like peptides are present in mammalian stomach and are selectively localized in nerve fibers in the gastric mucosa. Mammalian gastrin releasing peptide exists in two major forms, a 27 amino acid peptide and the C-terminal decapeptide of this larger peptide that contains the biologically active nonapeptide sequence. The 27 and 10 amino acid forms of GRP have similar gastrin-releasing activity, but larger exogenous doses of GRP decapeptide are needed because this shorter peptide is cleared more rapidly from the circulation than GRP-27.

The gastrin response to bombesin in dogs is enhanced by atropine and inhibited by bethanechol, whereas the gastrin response to food is not altered by the same doses of these agents. This pattern of response is reminiscent of the cholinergic inhibitory mechanism involved in neural stimulation of gastrin release in humans. In rat stomach bombesin stimulates both gastrin and somatostatin release. Atropine inhibits the somatostatin response in the perfused rat stomach. This inhibition could explain enhanced release of gastrin by bombesin after atropine treatment in vivo.

Effects of Opiates. Naloxone had no effect on basal gastrin or on gastrin response to an amino acid meal, whereas morphine significantly enhanced the gastrin response to the meal. Both agents inhibited the acid response to the meal. It was felt that naloxone probably inhibited an endogenous opioid-mediated mechanism that produces gastrin-independent stimulation of acid secretion, whereas the effects of morphine were more consistent with its known anticholinergic activity. The concept of an endogenous opiate acting to stimulate acid secretion was further supported by a study utilizing nalmefene, an orally administered opiate antagonist, in which a modest inhibition of acid secretion was obtained despite an enhanced gastrin response to a meal.

Intragastric Titration to Measure Acid Responses to Food. The studies of gastric secretion in which responses to various meals were determined all utilized the technique of intragastric titration, developed by John Fordtran. This technique utilized sodium bicarbonate to maintain intragastric pH at a constant level between 2.5 and 5.5. It was shown that the moles of base added to maintain constant pH was equal to the moles of acid secreted by the stomach. In subjects given a blended steak meal there was poor correlation between gastrin and acid responses, but the amount and nature of the meal were not varied. The increase in gastrin heptadecapeptide released by a mixed amino acid meal was compared with the increase required to produce a similar stimulation of acid secretion when gastrin-17 was infused intravenously, and it was concluded that endogenous stimulation of acid secretion by amino acids could be explained almost entirely by gastrin release.

The original intragastric titration method has been modified in our laboratory by use of automated syringe pump mixing and an autotitrator apparatus with computer monitoring of added base. By using dilute sodium hydroxide instead of sodium bicarbonate as the titrant, it is possible to titrate accurately at pH 7 or higher. We have utilized 5% glucose meals rather than saline meals for studies of gastric distention because glucose inhibits gastric emptying and is absorbed readily in the small intestine, whereas saline meals often cause diarrhea. We also use peptone as the standard protein stimulant because it is a reproducible soluble substance that is readily available.

Role of Circulating Gastrin in Gastric Acid Responses to Food. The contribution of gastrin to the total acid secretory response to meals and other stimulants of gastric acid secretion can be estimated by comparing the plasma gastrin concentrations achieved during stimulation with the plasma gastrin concentrations required to produce equivalent stimulation by intravenous infusions of gastrin. As discussed above, circulating big and little gastrins appear to have similar acid-stimulating activities, so total C-terminal immunoreactive gastrin provides a reasonable estimate of circulating gastrin bioactivity.

In comparing gastrin concentrations to acid secretion, it is important to study the dose response relationships over a wide range rather than at a single point. Such studies have revealed that circulating gastrin can account for most of the acid secretory responses to graded intragastric concentrations of peptone, confirming an earlier study showing that the amount of gastrin released by intragastric perfusion with a single dose of amino acid solution could explain most of the acid secretory response. There also was good general correlation between nutrients that cause gastrin release and gastric secretory response to each nutrient, except in the case of fat, which released modest amounts of gastrin but did not stimulate acid secretion.

When cephalic-vagal stimulation is added to pure gastric stimulation by simultaneous sham feeding and introduction of gastric meals, the acid responses are higher than can be explained purely by circulating gastrin. Cephalic vagal stimulation leads to acid secretory responses accompanied by very small increases in plasma gastrin and accentuates the acid response to meals without causing a corresponding enhancement of the plasma gastrin response. Thus the stimulatory action of the vagus nerve on the stomach is largely independent of gastrin responses.

The role of gastrin in mediating the acid secretory responses to combined stimulation by sham feeding and intragastric infusion of a homogenized meal was studied in normal volunteers. It was estimated that gastrin could account for about 90% of the measured acid responses in a group of 12 subjects. Individual variation was observed, possibly indicating different intestinal phase responses in these individuals.

Further proof of the role of gastrin in physiological regulation of acid secretion requires methods to neutralize or antagonize circulating gastrin. The gastrin receptor antagonists that have been available until the present time have not been effective for use in vivo. However, we have developed monoclonal antibodies capable of immunoneutralizing circulating gastrin and have shown in dogs that the gastric phase of acid secretion is almost completely gastrin-dependent (7). In rats, cephalic stimulation of acid secretion is almost completely independent of gastrin (8, 9). Similar studies cannot be done in humans until better gastrin antagonists become available.

Regulation of Gastrin Cell Function. Canine gastrin cells have been separated from somatostatin cells and studied in vitro in the presence of contaminating mucus cells. These isolated cells responded to bombesin and to amino acids. Bombesin receptors also have been shown directly on antral cells that had the characteristics of gastrin cells (10). These receptors have very high affinity, and maximal gastrin release often is obtained at 1 pM concentrations of bombesin. Adenosine was found to have dual actions on gastrin cells mediated by separate stimulatory and inhibitory receptors (11).

Regulation of Antral Gastrin Gene Expression. The relationship between gastrin release and gastrin synthesis can be studied by analyzing gastrin mRNA in experiments in which serum gastrin also is measured. The relationship between synthesis of gastrin and somatostatin also can be studied. Using standard methods to isolate mRNA from tissue, we found that prolonged inhibition of gastric secretion by omeprazole or by resection of the acid-secreting stomach in rats caused marked increase in gastrin mRNA and reciprocal decreases in somatostatin mRNA (12).

Somatostatin gene expression was found to increase during starvation and to decrease after refeeding (13). Gastrin mRNA decreases progressively during a 48–72 hour fast (14). When refeeding was carried out with specific nutrients, proteins and amino acids caused increases in gastrin mRNA within 15–30 min and decreases in somatostatin mRNA within 60 min. Fat caused modest increases in gastrin mRNA, whereas glucose had no effect.

Gastric Inhibitory Polypeptide and Neurotensin. Gastric inhibitory polypeptide and neurotensin are two gastrointestinal hormones released from their endocrine cells, located in proximal and distal intestine, respectively, by luminal fat. Gastrointestinal polypeptide was found to be a very weak inhibitor of pentagastrin-stimulated acid secretion in humans, and normal circulating concentrations did not approach the values required to inhibit acid secretion. Similarly, neurotensin was a weak inhibitor of meal-stimulated acid secretion in humans, and there is a wide gap between circulating concentrations after a fat meal and concentrations required to inhibit acid secretion.

Somatostatin Effects on Gastrin Release and Activity. Somatostatin is a peptide that can act as a circulating hormone or as a locally released paracrine peptide in the stomach. In rats there is good evidence that somatostatin can inhibit gastrin release by a paracrine action (15). When given parenterally, somatostatin is a more potent inhibitor of acid secretion than of gastrin release, and the effect of somatostatin 14 is much greater than that of somatostatin 28. The circulating form of somatostatin released by food in the intestine is mainly somatostatin 28, but somatostatin 14 is the form present in endocrine cells (D cells) in the body and antrum of the stomach.

The mechanism of action of somatostatin to antagonize gastrin and to inhibit acid secretion is not known, but it probably does not involve local release of prostaglandins. Somatostatin was not an effective releaser of prostaglandin from the perfused rat stomach, but a cholinergic agonist did cause release.

In humans, the ability of somatostatin to inhibit gastric acid secretion was not altered when endogenous prostaglandin synthesis was inhibited by indomethacin. Neither was the inhibition of gastric secretion by acidification of a meal, commonly felt to involve paracrine release of somatostatin, inhibited by indomethacin treatment (16). A more modern concept of somatostatin action to inhibit gastrin-stimulated acid secretion is that it acts in the gastric body to inhibit release of histamine from gastric enterochromaffin-like cells and that gastrin stimulates acid secretion largely by releasing histamine from the same cells. In the antrum, somatostatin can act directly on gastrin cells to inhibit gastrin release.

Although some studies suggest that the amount of somatostatin released into the circulation by fat may approach the physiological range needed for acid secretion, our own studies in humans do not support a role for circulating somatostatin in inhibition of acid secretion by fat (16). However, the possibility that various inhibitors of gastrin-stimulated acid secretion act by releasing gastric somatostatin to act in a paracrine fashion remains viable. We have used a potent monoclonal antibody to somatostatin to perform in vivo immunoneutralization studies (17). The ability of cholecystokinin to inhibit acid secretion is markedly impaired by prior treatment with this antibody (Lloyd, unpublished thesis).

Calcitonin Gene-Related Peptide Antagonizes Gastrin. Calcitonin gene related peptide (CGRP) is present in sensory nerve fibers innervating the gastric mucosa of most mammalian species. In urethane anesthetized rats, CGRP inhibits acid secretion stimulated by gastrin, histamine, cholinergic agonists, and CNS stimuli. It also inhibited pentagastrin-stimulated acid secretion in dogs. Subsequent studies showed that CGRP was a relatively specific inhibitor of gastrin-stimulated acid secretion in dogs. Of interest, calcitonin, the other product of the gene that produces CGRP, is a potent inhibitor of meal-stimulated acid secretion in humans.

Gastrin as a Cause of Clinical Gastric Acid Hypersecretion. The role of gastrin as the primary hormonal cause of gastric acid hypersecretion in the Zollinger-Ellison syndrome has been known since gastrin was isolated from Zollinger-Ellison tumors and gastrin radioimmunoassay was used to demonstrate high circulating gastrin concentrations in patients with this disease. One early observation that has contributed to the ease of diagnosis in patients with equivocal serum gastrin concentrations was that intravenous secretin often caused a paradoxical release of gastrin from gastrinomas. We also found that calcium infusion was a potent stimulant of gastrin release from gastrinomas. In 1973 gastrin and the Zollinger-Ellison syndrome were reviewed extensively.

In a few patients, hypergastrinemia associated with gastric acid hypersecretion originates from antral gastrin cells. Resected antrums from one small group of these patients were examined by quantitative immunohistochemistry with antibodies to gastrin and were found to have gastrin cell hyperplasia. A few patients with antral gastrin cell hyperfunction have been characterized physiologically and found to have exaggerated serum gastrin responses to meals and to bombesin but not to secretin. The initial patient to be identified with gastrin cell hyperfunction and acid hypersecretion was a technician in the Yalow and Berson laboratory (18).

Effects of Gastric Antisecretory Drugs on Gastrin Release. The first histamine H_2 receptor antagonist to be used in humans was metiamide. This drug was a potent inhibitor of acid secretion in patients with Zollinger-Ellison syndrome but did not affect the hypergastrinemia. Use of this type of drug was shown to be very effective for treatment of malignant hypergastrinemia in humans.

Another histamine H_2 receptor antagonist, cimetidine, was found to inhibit meal-stimulated gastric acid secretion profoundly without inhibiting gastrin release. In contrast, 16,16 dimethyl prostaglandin E_2 represented another class of acid inhibitors that inhibited acid secretion and also inhibited meal-stimulated gastrin release.

Gastrin in Peptic Ulcer Disease. No area of gastrin studies has been more difficult or controversial than defining the role of gastrin in ordinary peptic ulcer diseases. Many apparent differences between ulcer and control subjects, usually identified in small numbers of subjects in each group, have not been confirmed. It is now becoming clear that gastric infection with *Helicobacter pylori* in variable proportions of "normal control" subjects could have influenced the results markedly. Infection with this organism occurs in one third to two thirds of adults and leads to increased basal and stimulated serum gastrin concentrations that are not associated with increased gastric acid secretion. Almost all patients with duodenal ulcer are infected with *H. pylori*. None of our studies prior to the 1990s included knowledge of the status of infection with this bacterium.

Various studies have found that duodenal ulcer patients had higher serum gastrin values than normal control subjects, and an equivalent number of studies have concluded that there is no difference. In the largest comparison yet done, basal gastrin concentrations were found to be increased in duodenal ulcer patients, but gastrin responses to intragastric meals or to intragastric meals combined with sham feeding were found to be normal.

Sensitivity to Gastrin in Duodenal Ulcer. One of the earliest apparent differences identified between duodenal ulcer and "normal control" subjects was hypersensitivity to exogenous pentagastrin in duodenal ulcer patients. In a later study, gastric acid secretion was found to be abnormally sensitive to endogenous gastrin in duodenal ulcer patients, but only if acid responsiveness was expressed in an unusual way. Women were found to have higher serum gastrin concentrations and lower sensitivity to gastrin than men, possibly contributing to the known differences in peptic ulcer rates between women and men.

In another study, sensitivity to gastrin heptadecapeptide was compared in normal and duodenal ulcer subjects. Based on exogenous dose required to stimulate half maximal acid secretion, the ulcer patients appeared to be twice as sensitive as control subjects, but when circulating gastrin concentrations were measured they were not more sensitive. This discrepancy was explained by a twofold lower volume of distribution in the ulcer subjects, leading to twofold slower clearance of gastrin.

True hypersensitivity to gastrin has been found in cirrhotic patients with portacaval shunts. The explanation for this finding is unclear. The liver normally may remove from the circulation a natural antagonist of gastrin or the stomach may undergo hyperplasia of gastrin-responsive ECL cells after shunt.

Inhibition of Gastrin Release by pH in Ulcer Patients. In 1975 it was reported that inhibition of gastrin release at a low intragastric pH (2.5) was less pronounced in patients with duodenal ulcer than in control subjects. This observation has been difficult to confirm, even in our own hands (19), until recently. It now appears that insensitivity to low intragastric pH is found in subjects with *H. pylori* infection, regardless of ulcer status (6). Since the food-stimulated and bombesin-stimulated hypergastrinemia associated with *H. pylori* infection can be reversed by antibiotic eradication of the organisms (20), it will be interesting to compare pH inhibition profiles in infected subjects with and without peptic ulcer before and after *H. pylori* eradication.

Summary. The study of gastric physiology and pathophysiology has been aided enormously by the application of Yalow and Berson's radioimmunoassay methods to the measurement of gastrin. This hormone has proved to have a vital role in physiological control of gastric secretion and an important role in the pathological hypergastrinemias. The role of gastrin in peptic ulcer disease, and the interactions between gastrin and *H. pylori* infection, remain to be defined convincingly.

References

1. Walsh JH, Grossman MI. Gastrin. N Engl J Med 1975; 292:1324–1334, 1377–1384.
2. Walsh JH. Gastrointestinal Hormones. In: Johnson LR, ed. Physiology of the gastrointestinal tract, 2nd ed. New York: Raven Press, 1987.
3. Bunnett NW, Turner AJ, Hryszko J, Kobayashi R, Walsh JH. Isolation of Endopeptidase-24.11 (EC 3.4.24.11, "Enkephalinase") from the pig stomach. Gastroenterology 1988; 95:952–957.
4. Bunnett NW, Debas HT, Turner AJ, Kobayashi R, Walsh JH. Metabolism of gastrin and cholecystokinin by endopeptidase 24.11 from the pig stomach. Am J Physiol 1988; 255:G676–G684.
5. Lee SK, Thirlby RC, Thompson W, Walsh JH, Feldman M. Acute effect of experimental truncal vagotomy on serum gastrin concentrations. Ann Surg 1990; 211:136–140.
6. Tarnasky PR, Kovacs TOG, Sytnik B, Walsh JH. Impaired inhibition of meal-stimulated acid and gastrin secretion at low intragastric pH Helicobacter pylori infected normal subjects. Gastroenterology (Abstr) 1991; 100:A845.
7. Kovacs TOG, Walsh JH, Maxwell V, Wong HC, Axuma T, Katt E. Gastrin is a major mediator of the gastric phase of acid secretion in dogs: proof by monoclonal antibody neutralization. Gastroenterology 1989; 97:1406–1413.
8. Yang H, Wong H, Walsh JH, Taché Y. Effect of gastrin monoclonal antibody 28.2 on acid response to chemical vagal stimulation in rats. Life Sci 1989; 45:2413–2418.
9. Yanagisawa K, Yang H, Walsh JH, Taché Y. Role of acetylcholine, histamine and gastrin in the acid response

to intracisternal injection of TRH analog, RX 77368, in the rat. Regul Pept 1990; 27:161–170.

10. Vigna SR, Giraud AS, Mantyh PW, Soll AH, Walsh JH. Characterization of bombesin receptors on canine antral gastrin cells. Peptides 1990; 11:259–264.
11. Schepp W, Soll AH, Walsh JH. Dual modulation by adenosine of gastrin release from canine G-cells in primary culture. Am J Physiol 1990; 259:G556–G563.
12. Wu SV, Giraud A, Mogard M, Sumii K, Walsh JH. Effects of inhibition of gastric secretion on antral gastrin and somatostatin gene expression in rats. Am J Physiol 1990; 258:G788–G793.
13. Wu SV, Sumii K, Tari A, Mogard M, Walsh JH. Regulation of Gastric Somatostatin Gene Expression. Metabolism 1990; 39, No. 9, Suppl. 2:125–130.
14. Wu V, Sumii K, Tari A, Sumii M, Walsh JH. Regulation of rat antral gastrin and somatostatin gene expression during starvation and after refeeding. Gastroenterology 1991;101:1552–1558.
15. Yang H, Wong H, Wu SV, Walsh JH, Taché Y. Somatostatin monoclonal antibody immunoneutralization increases gastrin and gastric acid secretion in urethane-anesthetized rats. Gastroenterology 1990; 99:659–665.
16. Mogard MH, Maxwell V, Wong H, Reedy TJ, Sytnik B, Walsh JH. Somatostatin may not be a hormonal messenger of fat-induced inhibition of gastric functions. Gastroenterology 1988; 94:405–408.
17. Wong HC, Walsh JH, Yang H, Taché Y, Buchan AM. A monoclonal antibody to somatostatin with potent in vivo immunoneutralizing activity. Peptides 1990; 11:707–712.
18. Berson SA, Walsh JH, Yalow RS. Radioimmunoassay of gastrin in human plasma and regulation of gastrin secretion. In: Anderson S, ed. Nobel Symposium XVL: Frontiers in Gastrointestinal Hormone Research. Stockholm, Sweden: Almquist and Wilsell, 1973:57–68.
19. Karnes WE, Berlin RG, Maxwell V, Sytnik B, Root JK, Walsh JH. Prolonged inhibition of acid secretion causes hypergastrinaemia without altering pH inhibition of gastrin release in humans. Aliment Pharmacol Therap 1990; 4:443–456.
20. Graham DY, Opekun A, Lew GM, Klein PD, Walsh JH. Helicobacter pylori-Associated Exaggerated Gastrin Release in Duodenal Ulcer Patients. The Effect of Bombesin Infusion and Urea Ingestion. Gastroenterology 1991; 100:1571–1575.

Rosalyn S. Yalow at her desk.

The Brooklyn Gastrinomas

Eugene Straus, M.D., and Jean-Pierre Raufman, M.D.

Abstract

During the past seven years we have diagnosed and treated 12 patients with the Zollinger-Ellison syndrome (ZES), some of whom have had virtually unique manifestations. Among these are a 74-year-old man who was cured after endoscopic removal of a submucosal duodenal gastrinoma, suggesting the usefulness of an operative approach using intraoperative endoscopic transillumination; a 33-year-old man in whom the manifestations of ZES brought him into conflict with the law; and a 10-year-old domestic cat who was cured surgically, the gastrinoma providing material for the first determination of the sequence of cat gastrins.

The three cases presented here provide important lessons with regard to the natural history of gastrin-secreting tumors and their management, the impact of symptoms and the availability of health care on behavior and society, and the comparative biology of hormone-secreting tumors. Two of these cases have been reported in greater detail elsewhere (1, 2). The broad range of interest illustrated by these cases is a fitting reflection of the career of Rosalyn Sussman Yalow, who has contributed so greatly in so many areas, including the understanding of tissue and plasma gastrins.

Case 1

The patient is a 74-year-old man who was first seen by us in May 1983 when he complained of several years of epigastric burning pain. He had no other symptoms. Twenty-five years earlier he had undergone a partial gastrectomy with vagotomy and Billroth II reconstruction for duodenal ulceration. He had then been asymptomatic for nearly 22 years.

The patient's symptoms improved quickly after institution of treatment with cimetidine 300 mg four times daily. Endoscopic examination revealed six small intestinal ulcerations at the gastroenterostomy site. A basal acid output (BAO) was determined to be >5 mEq/h. Fasting plasma gastrin was 450 pg/mL (normal, 41.7 ± 4.6 pg/mL [mean ± SEM]). Intravenous injection of secretin resulted in plasma gastrin concentrations of 625, 700, 650, 575, 450, and 400 pg/mL at 1, 5, 10, 15, 30, and 45 minutes, respectively.

A diagnosis of ZES was made. CAT scan revealed no evidence of tumor. Exploratory surgery was declined by the patient.

During the 8 years following the diagnosis of ZES, the patient was asymptomatic on treatment with H2-receptor antagonists and finally omeprazole (60 mg daily). In December 1990, routine endoscopic examination revealed a 1-cm polypoid mass in the afferent limb of the Billroth II anastomosis. Histopathologic examination indicated that the tumor was of endocrine type. Fasting plasma gastrin was then 300 pg/mL. A 1.0-cm polypoid mass with a reddened, irregular surface was found adjacent to the ampulla of Vater. The polyp was removed endoscopically using a wire snare and electrocautery. Multiple biopsies were taken from the edge of the polypectomy site to ascertain if any residual tumor was present. Histologic examination of the polyp revealed a submucosal neuroendocrine tumor with positive immunohistochemical staining for gastrin. Six

---

From the Division of Digestive Diseases, State University of New York-Health Science Center at Brooklyn, Brooklyn, NY. Address reprint requests and correspondence to Eugene Straus, M.D., SUNY/HSCB, Box 1196, 450 Clarkson Avenue, Brooklyn, NY 11203-2098.

hours after the polypectomy the patient complained of abdominal pain. The following morning the patient had a temperature of 101.5°F, and X-rays of the abdomen revealed free air under the diaphragm. Abdominal pain increased over the next 24 hours and the patient was taken to the operating room, where a 1.5-cm full thickness perforation in the second portion of the duodenum was oversewn. The postoperative course was uneventful. A repeat secretin test demonstrated normal fasting plasma gastrin of 50 pg/mL with no changes in concentration following injection of secretin. The patient was taken off all medications and has been well during a 6-month follow-up period.

Discussion. ZES caused by single or multiple gastrinomas of the duodenal wall has been well established (3–12). Recent reports have observed a higher frequency of duodenal gastrinomas than had been generally appreciated (3), and suggest the possibility that virtually all cases of ZES in patients with multiple endocrine neoplasia type 1 (MEN-1) result from duodenal gastrinomas (6). Nevertheless, these tumors have rarely been localized endoscopically (7–11), and only one well-documented cure of ZES resulting from endoscopic removal of a duodenal gastrinoma has been previously reported (8).

Duodenal gastrinomas tend to be small and generally are undetected by CAT scan (3, 7–14). Frucht et al. have suggested routine endoscopic transillumination of the duodenum in all patients with ZES who undergo exploratory laparotomy in the attempt to remove gastrinomas. Their study excluded all patients with MEN-1 because of their belief that these patients generally have multiple microscopic pancreatic tumors which are not surgically curable (3). None of the 12 duodenal gastrinomas found at surgery was seen during prior duodenoscopy.

Although few submucosal duodenal gastrinomas have been described endoscopically, our patient's tumor was typical of the sessile mound with central depression previously called "volcano" or "donut-like" (7–9). These tumors may appear in any portion of the duodenum (3, 5–14). The demonstration that in some cases duodenal gastrinomas can be localized endoscopically suggests that careful examination of the duodenum using colonoscopes which can reach beyond the ligament of Treitz should be considered in patients with ZES.

The current case raises the question whether endoscopic localization of a duodenal gastrinoma should provoke attempts at endoscopic extirpation of the tumor. Although they tend to be small tumors, their submucosal location and the requirement for complete removal, along with our experience in this case, suggest that an operative approach may be required. In addition, intraoperative endoscopic transillumination may demonstrate additional tumors.

Case 2

We first saw this patient on the Prison Ward at Kings County Hospital Center (KCHC) in April 1985. He was then 33 years old and complained of severe abdominal pain. He had an 8-year history of peptic ulcer diseases. Seven years earlier a perforated duodenal ulcer had been oversewn at KCHC. He had recently been experiencing hematemesis and melena, but epigastric abdominal pain and episodes of diarrhea had persisted since his prior surgery, and he was aware that these responded to treatment with cimetidine.

The diagnosis of ZES was immediately entertained. Esophagogastroduodenoscopy (EGD) demonstrated 4+ peptic esophagitis, hypertrophic gastric folds with increased amounts of gastric fluid, a deformed, edematous pyloric channel, a duodenal bulb with a 0.7-cm ulcer with a deep necrotic base, multiple (4–5) 0.3–0.4 cm ulcers, and duodenitis of the second portion of the duodenum. Fasting plasma gastrin was 1000 pg/mL, with a rise to 2000 pg/mL 2 minutes after intravenous secretin injection.

This patient had been arrested for armed robbery and was being kept in the Brooklyn House of Detention awaiting a hearing when his ulcer disease led to the transfer to KCHC. With regard to the robbery, he claimed that unremitting abdominal pain and consequent sleeplessness resulted in his robbing an elderly woman for money to buy cimetidine and antacids. This story, along with supporting evidence from us relating to his ZES, was enough for a judge to subsequently dismiss the charges against him. He had no previous arrest record.

The patient was discharged on ranitidine 750 mg and probanthine 30 mg every 6 h. On this regimen he secreted gastric acid at a rate of 0.1 mEq/h for the 5th–6th hour after medication. He did very well until April 1985, when he was shot in the back "while waiting for the light to turn green." He was taken to surgery emergently, and repair was made to the stomach, duodenum, and inferior vena cava. No attempt was made to localize a gastrinoma. He recovered very rapidly, and continued to do well with respect to his ZES on H-2 blocker therapy. In December 1985 he was admitted to the heroin detoxification unit at KCHC, where he remained for about one month before moving into a halfway house in Brooklyn.

In April 1986 the patient complained of abdominal pain and diarrhea in spite of compliance with his treatment regimen. EGD revealed a 1.5-cm bulbar ulcer, and his gastric acid secretory rate was 75 mEq/h. At that time omeprazole 80 mg daily therapy began. The secretory rate was reduced to 6 mEq/h, and the ulcer healed. Interestingly, his fasting plasma gastrin concentrations have consistently been below 200 pg/mL since his surgery for the gunshot wound. Nonetheless, secretin tests continued to be positive, with increases over fasting concentrations of 300–500 pg/mL within 5 minutes after injection on three separate occasions. Repeated efforts to image a gastrinoma with CAT scans, MRI, and angiograms have been unsuccessful.

Discussion. During the past seven years this patient has been free of drugs and alcohol. He has become an expert in computer applications, has maintained a very responsible position with a law firm, and was recently married. He is a reliable patient who pays close attention to his medical condition. It would therefore appear that he was indeed driven to armed robbery by his ignorance of other means of obtaining costly medications. He was caught trying to return the looted handbag to his victim. Had he not been admitted to the KCHC Prison Ward, he might have continued to go undiagnosed, and gone on to prison. The hazards of street life in his neighborhood also resulted in a gunshot wound which clearly caused infarction and necrosis of a major portion of the gastrinoma tissue which probably resides within the wall of his duodenum.

Case 3

A 10-year-old female cat presented with a one-month history of vomiting liquid and undigested food, associated with weight loss. EGD revealed "irregularity in the duodenum" and suspicion of a duodenal mass. She was given cimetidine 50 mg orally twice per day, and this rapidly resulted in improved appetite and cessation of vomiting. On the eighth hospital day a laparotomy was performed. Preoperative fasting serum gastrin concentration was 350 pg/mL (normal for cats in our laboratory, 40 ± 3 pg/mL [mean ± SEM, n = 5]). At surgery the duodenum was found to be severely inflamed and ulcerated. A small tumor (7-mm diameter) was resected from the middle of the right limb of the pancreas. Postoperative serum gastrin was 30 pg/mL. The cat was noted to recover rapidly, with an immediate increase in appetite. Following three days of intravenous cimetidine and three days of oral cimetidine, all medications were withdrawn and she has been well for more than one year.

The gastrinoma removed from this cat contained 6.5 mg gastrin-IR/gm of wet weight. Fractionation of the MB C18 column yielded 4 peaks of gastrin-IR, corresponding to sulfated and unsulfated G17 and sulfated and unsulfated G34. The ratio of G17/G34 was approximately 70/30.

Discussion. Since our initial description of canine Zollinger-Ellison syndrome (15), there have been a number of reports of gastrinomas in both dogs and cats (16, 17). The diagnosis has generally been made at necropsy. This cat was restored to normal health after the removal of a pancreatic gastrinoma.

The gastrin content of the tumor was exceptionally high. While gastrinoma tissue may contain higher concentrations of gastrin than that found in normal gastrointestinal mucosa (19), tumor gastrin concentrations are generally in the microgram per gram of wet weight range. This tumor contained 6.5 mg/gm of wet weight, thereby facilitating the analytical studies. Nevertheless, gastrin peptides have been purified and sequenced from single antra of dog and goat stomach (20).

The sequence of cat G17 has been published by Agarwal et al. (18). They found that cat G17 differs from dog and human by an L (Leu) for M (Met) substitution in position 5 of the former, and an A (Ala) for E (Glu) substitution in position 10 of the latter (Fig.). The present data confirm the sequence of cat G17.

The sequence of cat G34 was determined for the first time in studies of this cat's tumor. These studies indicated that cat G34 has an additional sequence variation in comparison to dog, in that cat has Q (Gln) in position 10. Sequence variations are greater in G34 peptides from human, pig, and goat (Fig.).

The COOH-terminal pentapeptide, which contains gastrin's full biologic activity and potency, is identical in all mammalian species. Sequence variation tends to be greater within the NH_2-terminal half (1–17) of the peptide. Nonetheless, the most striking species-specific gastrin sequence variations are the D (Asp) for G (Gly) substitution within the COOH-terminal pentapeptide of chicken gastrin, and the amino-acid deletions in chinchilla, guinea pig, and opossum gastrins (Fig.).

Species variations in the sequence of a specific hormonal peptide, such as gastrin, do not

	.	.	.	.	▼	.	.	.	.	▼	.	.	.	.	▼	.	.	.	.	▼	.	.	.	.	▼	.	.	.	.	▼	.	.	.	.	▼
CAT	<E	L	G	L	Q	G	P	P	Q	Q	V	A	D	L	S	K	K	Q	G	P	W	L	E	E	E	E	A	A	Y	G	W	M	D	F	
DOG	<E	L	G	L	Q	G	P	P	Q	**L**	V	A	D	L	S	K	K	Q	G	P	W	**M**	E	E	E	E	A	A	Y	G	W	M	D	F	
GOAT	<E	L	G	L	Q	**D**	P	P	**H**	**M**	V	A	D	L	S	K	K	Q	G	P	W	**V**	E	E	E	E	A	A	Y	G	W	M	D	F	
PIG	<E	L	G	L	Q	G	P	P	**H**	**L**	V	A	D	L	**A**	K	K	Q	G	P	W	**M**	E	E	E	E	**E**	A	Y	G	W	M	D	F	
MAN	<E	L	G	**P**	Q	G	P	P	**H**	**L**	V	A	D	**P**	S	K	K	Q	G	P	W	L	E	E	E	E	**E**	A	Y	G	W	M	D	F	
OPOSSUM	<E	L	G	**P**	Q	**D**	**L**	P	**Y**	**L**	**T**	A	D	L	S	K	K	Q	G	P	W	L	E	E	E	E	_	A	Y	G	W	M	D	F	
CHINCHILLA	<E	L	**E**	**P**	Q	G	P	P	**H**	**L**	**G**	**T**	D	L	S	K	K	Q	G	P	W	**A**	E	E	E	_	A	A	Y	G	W	M	D	F	
GUINEA PIG	<E	L	G	**P**	Q	**V**	P	**A**	**H**	**L**	**R**	**T**	D	L	S	K	K	Q	G	P	W	**A**	E	E	E	_	A	A	Y	G	W	M	D	F	

Fig. Amino acid sequences of cat and other mammalian gastrins.

necessarily parallel phylogeny; for example, rhesus monkey gastrin is identical to dog, whereas rhesus monkey insulin, glucagon, pancreatic polypeptide, and VIP are identical to human (21).

References

1. Straus E, Raufman J-P, Samuel S, Waye JD, Jensen RT. Endoscopic cure of the Zollinger-Ellison syndrome. (in press)
2. Eng J, Du B-H, Johnson GF, Kanakamedala S, Samuel S, Raufman J-P, Straus E. Cat gastrinoma and the sequence of cat gastrins. Regulatory Peptides 1992; 37:9–13.
3. Frucht H, Norton JA, London JF, et al. Detection of duodenal gastrinomas by operative endoscopic transillumination. Gastroenterology 1990; 90:1622–1627.
4. Zollinger RM, Ellison EH. Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. Ann Surg 1955; 142:709–728.
5. Straus E. Ulcerogenic endocrine tumors. In: Berk JE, ed. Bockus Gastroenterology, 4th ed. W.B. Saunders Company, 1984; 7:4467–4479.
6. Pipellers-Marichal M, Somers G, Willems G, et al. Gastrinomas in the duodenums of patients with multiple endocrine neoplasia type 1 and the Zollinger-Ellison Syndrome. NEJM 1990; 322:723–727.
7. Wu WC, Kengis J, Whalen G, Schulte WJ, Unger GF, Classen M. Endoscopic localization of a duodenal wall tumor in Zollinger-Ellison syndrome. Gastroenterology 1974; 66:1237–1239.
8. Gilhool WJ. Endoscopic diagnosis and removal of a duodenal wall gastrinoma. Am J Gast 1984; 79:679–683.
9. Wadas DD, Foutch PG, Manne RK, Sanowski RA. Endoscopic diagnosis of a duodenal gastrinoma. Gastrointestinal Endoscopy 1988; 34:430–431.
10. Piccione PR, Hamilton R, Kotler DP, Scholes J, McCray RS. The endoscopic localization of a gastrinoma in the afferent loop of a gastrojejunostomy. Gastrointestinal Endoscopy 1988; 34:439–440.
11. Donovan DC, Dureza R, Jain U. Gastrinoma of the duodenum. Diagnosis by endoscopy. NYSJM 1979; 00:1766–1768.
12. Hoffmann JW, Fox PS, Wilson SD. Duodenal wall tumors and the Zollinger-Ellison Syndrome. Arch Surg 1973; 107:334–339.
13. Antonioli DA, Dayal Y, Dvorak AM, Banks P. Zollinger-Ellison syndrome. Cure by surgical resection of a jejunal gastrinoma containing growth hormone releasing factor. Gastroenterology 1987; 92:814–823.
14. Oberhelman HA. Excisional therapy for ulcerogenic tumors of the duodenum. Arch Surg 1972; 104:447–453.
15. Straus E, Johnson GF, Yalow RS. Canine Zollinger-Ellison syndrome. Gastroenterology 1977; 72:380–381.
16. Happe RP, van der Gaag I, Lamers CBHW, van Toorenburg J, Rehfeld JF, Larsson LI. Zollinger-Ellison syndrome in three dogs. Vet Pathol 1980; 17:177–186.
17. Middleton DJ, Watson ADJ. Duodenal ulceration associated with gastrin-secreting pancreatic tumor in a cat. JAVMA 1983; 183:461–462.
18. Agarwal KL, Kenner GW, Sheppard RC. Feline gastrin: an example of peptide sequence analysis by mass spectrometry. J Am Chem Soc 1969; 91:3096–3097.
19. Greenstein RJ, Claine DJ, Straus E, Yalow RS. Distribution of immunoreactive gastrin in a patient with metastatic gastrinoma. Am J Gastro 1981; 82:886–889.
20. Bonato C, Eng J, Hulmes JD, Miedel M, Pan Y-CE, Yalow RS. Sequences of gastrins purified from a single antrum of dog and of goat. Peptides 1986; 7:689–693.
21. Yu J, Xin Y, Eng J, Yalow RS. Rhesus monkey gastroenteropancreatic hormones: relationship to human sequences. Regulatory Peptides 1991; 32:39–45.

Studies of a Proposed Mechanism Regulating Parietal Cell Emptying

GÖRAN NILSSON PH.D., BENGT ROMELL, MSC., KINFE GIRMA, V.MD., AND REIN SEENSALU M.D., PH.D.

ALTHOUGH the mechanisms by which acid production of the parietal cell becomes activated are relatively well known, the mechanism for emptying of the parietal cell does not seem to have been studied as extensively. Several studies indicate a delay in onset of acid secretion. Two studies (1, 2) have shown that the delay is not due to a slow or late release of gastrin or histamine, and is therefore probably caused within the parietal cell.

Activation of parietal cells initiates morphologic change in the cells and activates the mechanism for secretion of hydrogen ions. Considering the known delay in onset, we have advanced this hypothesis: Initially, the acid produced is prevented from leaving the cell. During this period the strong acid is formed within the parietal cell. In the absence of acid in the stomach other physiological events take place there. When the appropriate conditions exist, the acid leaves the cell, enters the gastric cavity, and exerts its effects there.

Histamine plays an important role in the activation of the parietal cell by stimulating membrane H_2 receptors. Possibly, histamine also acts in some way to prevent the acid from leaving the cell immediately. To test this hypothesis, we incubated isolated gastric glands or cells from rabbits with labelled aminopyrine (AP). Following intracellular accumulation of the AP, attempts were made to expel it from the parietal cells. Assuming that the delay in secretion may be due to activation of membrane H_1 receptors, we administered H_1-receptor antagonists (H_1RAs) to the incubation medium to counteract the postulated action of histamine and study the characteristics of the effects obtained.

Materials and Methods

AP is uncharged in a neutral solution and it will thus cross the membrane of the parietal cell. Intracellularly, it will reach the acid compartment of the cell, where it becomes charged and therefore trapped. This forms the basis for the use of the AP technique to measure the acid content of the parietal cell (3). We have extended the use of this technique by using it to indicate the emptying of the parietal cell. Glands (4) or cells (5) were prepared according to the techniques described elsewhere.

The preparations used have been investigated under basal conditions and following stimulation with histamine dihydrochloride alone or in combination with the phosphodiesterase inhibitor IMX, with db cAMP or with the stable choline ester carbachol. Following accumulation of AP, different H_1- or H_2-receptor antagonists were added to the system, and the reduction in cellular AP content was determined. Oxygen consumption in the system was determined by the Warburg technique.

Results and Discussion

When H_1-RAs were added to histamine-stimulated gastric glands, several, but not all, H_1RAs expelled the previously accumulated AP effectively and in a dose-related way (Fig. 1). A number of subsequent studies were performed to elucidate the specificity of the effects observed.

H_1RAs are known to exert anticholinergic side-effects and also to act as local anesthetics by

---

From the Department of Physiology, Swedish University of Agricultural Sciences, Uppsala, and Research Center, Huddinge University Hospital, The Karolinska Institute, Huddinge, Sweden. Address reprint requests to Dr. Göran Nilsson, Professor in Physiology, Department of Physiology, The Swedish University of Agricultural Sciences, Box 7045, SLU, 750 07 Uppsala, Sweden.

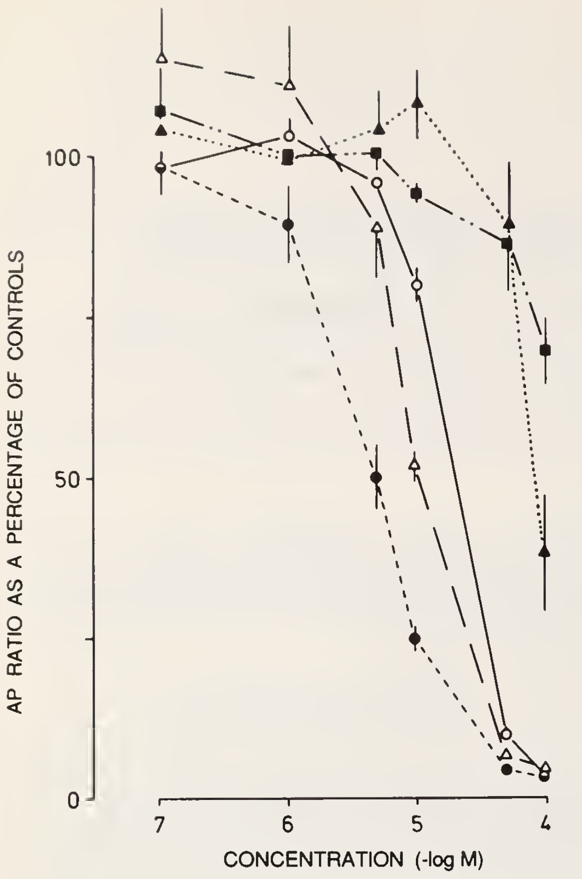


Fig. 1. Effects on AP content of glands prestimulated with histamine (10^{-5} M) for 30 min, then exposed to histamine (10^{-5} M) with H_1RAs clemastine (●), promethazine (△), hydroxyzine (○), cetirizine (■) or tripelennamine (▲) for another 30 min. AP ratios expressed as percentage of AP ratios for glands stimulated with histamine (10^{-5} M) for 60 min (n = 5).

influencing cell-membrane stability. However, exposure of histamine-activated glands to a wide range of concentrations of atropine or lidocaine did not result in expulsion of the accumulated AP.

Other experiments were performed to determine whether exposure to H_1RAs influence viability of gastric glands, which might result in leakage of accumulated AP. Incubation of gastric glands with trypan blue is a widely used method to determine whether parietal cells are alive. No difference was found between exposed and untreated glands. When the oxygen consumption of the glands was determined, only an insignificant reduction was found following treatment with the antagonists. In other experiments performed to illustrate the viability of the parietal cells, glands were first stimulated with histamine. An H_1RA was then added and incubation was continued. Finally, increased concentrations of histamine or db cAMP were added to stimulate the reuptake of

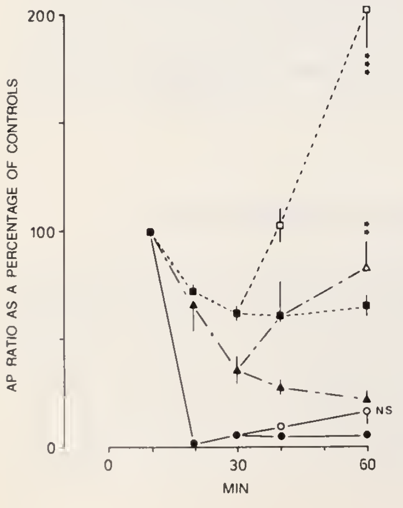
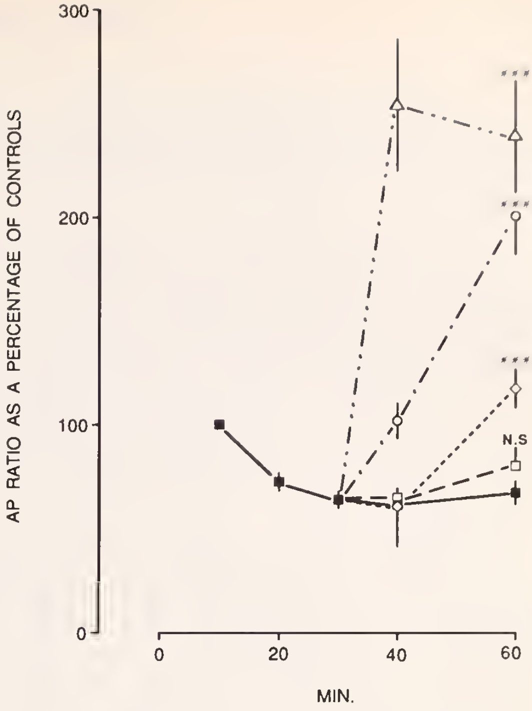


Fig. 2. Effects on AP content of glands prestimulated with histamine (5×10^{-6} M) for 10 min, exposed to histamine (5×10^{-6} M) with hydroxyzine (10^{-5} M ■, 2×10^{-5} M ▲ or 5×10^{-5} M ●) for 20 min, then exposed to db cAMP (3.2×10^{-4} M) stimulation for another 30 min (white symbols). AP ratios expressed as percentage of AP ratios for glands stimulated with histamine (5×10^{-6} M) at corresponding times (n = 6).

Fig. 3. Effects on the AP content of unstimulated glands and glands stimulated with histamine (10^{-5} M) or db cAMP (3.2×10^{-4} M) for 30 or 20 min respectively, then exposed to either hydroxyzine (10^{-7}–10^{-4} M; ◆), histamine (10^{-5} M) with hydroxyzine (10^{-7}–10^{-4} M ■), or db cAMP (3.2×10^{-4} M) with hydroxyzine (10^{-7}–10^{-4} M ●) for another 30, 30, or 20 min. AP ratios expressed as percentage of AP ratios for unstimulated glands, histamine (10^{-5} M) or db cAMP (3.2×10^{-4} M) stimulated glands at corresponding times (n = 5).



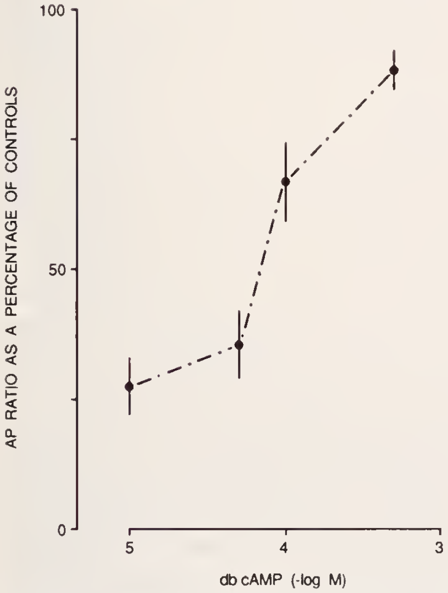
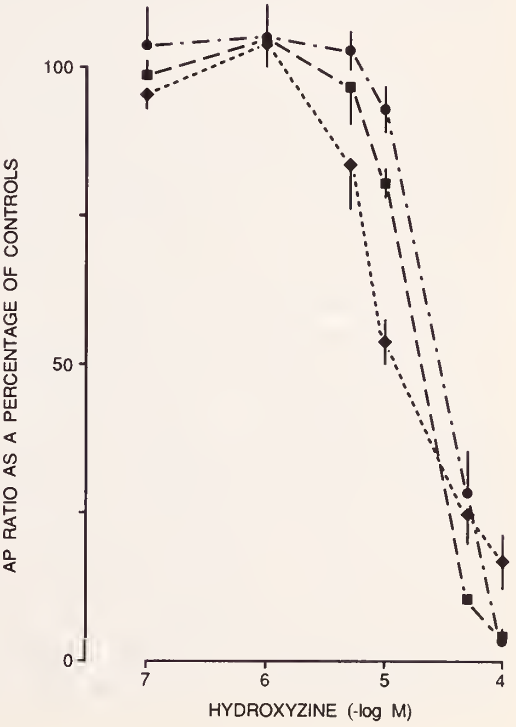


Fig. 4. Effects on the AP content of glands prestimulated with db cAMP (10^{-5} – 5×10^{-4} M) for 20 min, then exposed to db cAMP (10^{-5} – 5×10^{-4} M) with hydroxyzine (2×10^{-5} M) for another 20 min. AP ratios expressed as percentage of AP ratios for glands stimulated with db cAMP (10^{-5} – 5×10^{-4} M) for 40 min (n = 5).

Fig. 5. Effects on the AP content of glands prestimulated with histamine (5×10^{-6} M) for 10 min, then exposed to histamine (5×10^{-6} M) with hydroxyzine (10^{-5} M ■) for 20 min, and finally exposed to increased stimulation by histamine (10^{-4} M □), db cAMP (3.2×10^{-4} M ○), papaverine (10^{-4} M ◇), or IMX (10^{-4} M △) for another 30 min. AP ratios expressed as percentage of AP ratios for glands stimulated with histamine (5×10^{-6} M) at corresponding times (n = 5).



expelled AP. Histamine only slightly increased the AP uptake (not shown), whereas the uptake was large following the addition of db cAMP (Fig. 2).

The results of the experiments described above thus indicate that several H_1RAs have the ability to expel accumulated AP from parietal cells, and that this elimination is not due to any of the tested pharmacologic side-effects of the antagonists or to reduced viability of the cells, as indicated by their oxygen consumption and their ability to become restimulated following the addition of db cAMP.

Subsequent studies were undertaken to further elucidate the mechanism of action of the H_1RAs. Attempts were made to determine (a) whether the AP-expelling effect is exerted via membrane receptors or intracellularly, (b) whether such effects are found in preparations of mucosal cells as well as in glands and in gastric preparations from species other than rabbit, and (c) whether substances other than H_1RAs can evoke elimination of accumulated AP.

Attempts to Localize Site of Action of H_1 Receptor Antagonists. Some studies were performed to compare effects exerted by H_1- and H_2-receptor antagonists on gastric glands. When antagonists were added following preincubation with histamine, the intracellular AP content was only slightly reduced by the H_2-receptor antagonist (H_2RA) ranitidine, whereas the H_1RA hydroxyzine almost completely eliminated the accumulated AP. When the antagonists were added simultaneously with the histamine, AP uptake was prevented by both antagonists. However, whereas the histamine-induced increase in oxygen consumption was almost abolished following exposure to ranitidine, it was only slightly lowered by hydroxyzine. The results thus indicate that H_1- and H_2-receptor antagonists influence the AP content of gastric glands differently and that the AP-expelling effects of H_1RAs are not exerted via effects on H_2 receptors.

The observation that some H_1RAs, but not others, significantly expel accumulated AP (Fig. 1) may suggest that the expelling effects are not mediated through H_1 receptors either. It is instead possible that H_1RAs interfere with the H_3 receptor or some other so far unidentified histamine receptor on the parietal cells. If so, the less effective H_1RAs may act essentially as H_1RAs and therefore are unable to interfere with other histamine receptors. The observation that further addition of histamine did not significantly reactivate the AP uptake may, however, mean that the H_1RAs do not interfere with histamine membrane receptors at all. Instead, the effect may be exerted intracellularly. The difference in effects between the various H_1RAs may therefore be due to different physicochemical properties of the drugs, permitting some, but not all, of them to cross the cell membrane and exert their effect intracellularly.

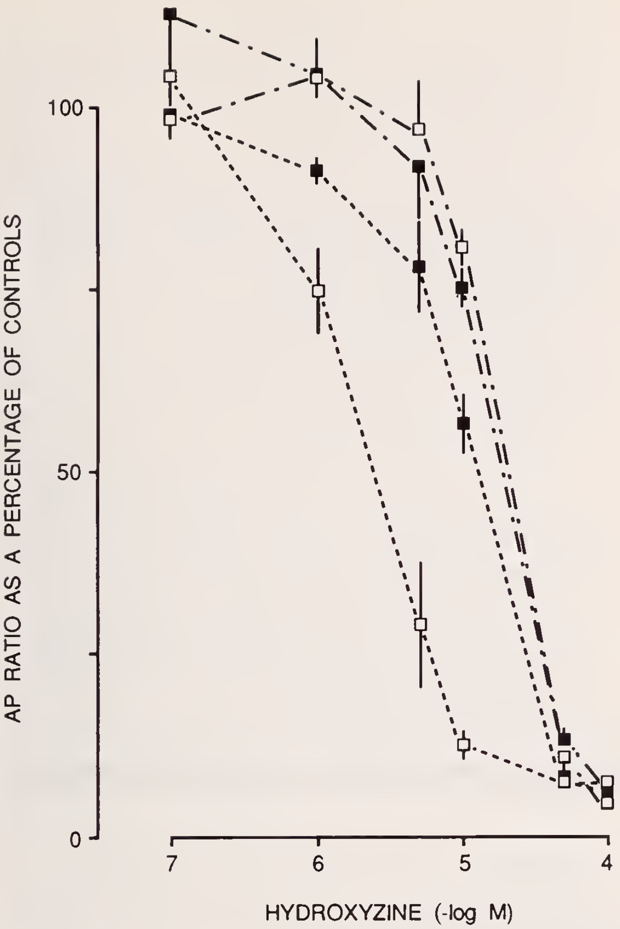
Studies by others (6) have shown that H_1RAs may reduce the increase of intracellular cAMP following stimulation of the gastric mucosa. However, since the increase in intracellular cAMP in our experiments is produced before the antagonists are added, the antagonists probably also interfere with already formed cAMP.

In further studies we have therefore investigated the interaction between H_1RAs and cAMP. When glands were activated by db cAMP, H_1RAs eliminated AP in a similar way to that following histamine activation (Fig. 1). However, on a molar basis, the H_1RAs were more efficient in eliminating AP in histamine-activated than in db cAMP-activated glands, and they were still more efficient in reducing the AP content of unstimulated glands (Fig. 3). There is undoubtedly less cAMP activation in basal glands than in histamine-stimulated glands, and still more cAMP acts on the gland following db cAMP stimulation. If H_1RAs interfere with the effect of cAMP, it is therefore logical that the smallest AP elimination occurred on stimulation with db cAMP and the largest elimination in unstimulated glands.

Further evidence for interference with cAMP was found in experiments in which glands were activated by various concentrations of exogenous db cAMP and the same dose of the H_1RA hydroxyzine was given. Also in these studies, elimination of AP was inversely correlated to the concentration of db cAMP in the system (Fig. 4).

Experiments were also performed to determine whether significant restimulation of glands could be achieved by other means than db cAMP. In all studies in which the intracellular cAMP concentration was raised by preventing the effect of the phosphodiesterase, AP uptake was reactivated (Fig. 5). Taken together, results described above thus suggest that H_1RAs eliminate accumulated AP by interfering with the action of intracellular cAMP.

Studies of Paracrine Influences on AP-Expelling Mechanism. To determine whether paracrine mechanisms may be involved when H_1RAs eliminate accumulated AP from parietal cells, preparations of glands or cells were stimulated with histamine and an H_1RA was then added. As



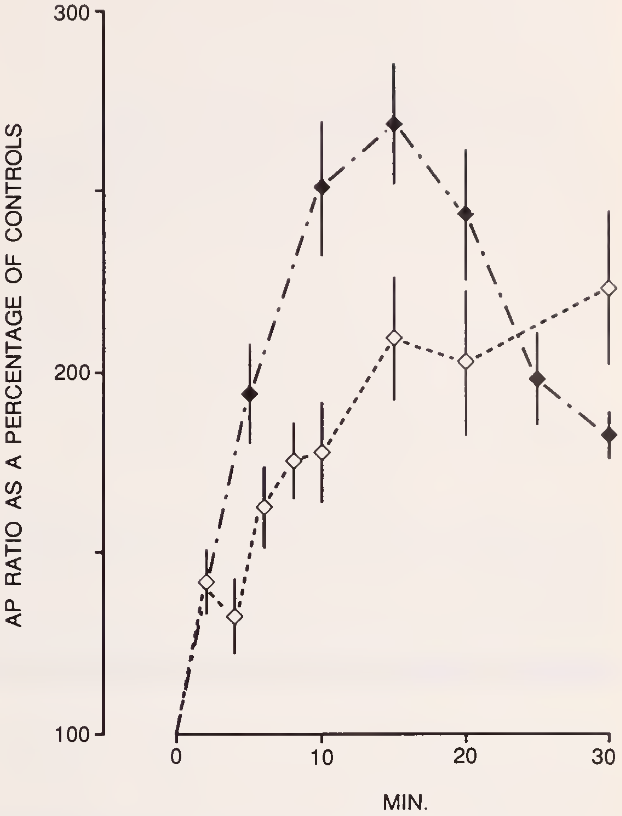


Fig. 6. Left Effects of hydroxyzine (10^{-7}–10^{-4} M) on accumulated AP of histamine-stimulated rabbit gastric glands (—·—·) and cells (------). After 30 min of prestimulation with histamine (10^{-5} M), hydroxyzine was added and incubation continued with hydroxyzine and histamine (10^{-5} M) for 10 (■) or 30 min (□). AP ratio expressed as percentage of AP ratios of glands or cells stimulated with histamine (10^{-5} M) for 40 and 60 min respectively (n = 5). Fig. 7. Right AP accumulation in glands (♦) and cells (◇) stimulated with carbachol (5×10^{-5} M). After 30 min of basal preincubation, carbachol was added and change in AP accumulation was observed during following 30 min. AP ratios for glands or cells stimulated with carbachol expressed as percentage of AP ratios for unstimulated glands or cells at corresponding times (n = 10).

with glands, the AP content was readily eliminated from gastric cells (Fig. 6). As a matter of fact, lower ED_{50} values were found for the antagonists with preparations of cells, which may indicate that some paracrine mechanism is involved in the process of retaining the accumulated AP within the secretory canaliculi, that is, the further digestion of the gastric glands to obtain a preparation of mucosal cells may have destroyed this mechanism.

Elimination of accumulated AP was also shown in preparations of pig gastric mucosa, demonstrating that this phenomenon also occurs in species other than rabbit.

Elimination of Accumulated AP by Carbachol. To find out if substances involved in the physiologic activation of parietal cells influence elimination of accumulated AP, we have studied the effects of the antral hormone gastrin and a stable choline ester, carbachol. The results obtained so far indicate that cholinergic impulses may play a role in parietal cell emptying. When gastric glands are incubated with carbachol it has been shown that there is an initial uptake of AP, followed after 10–15 min by a reduction in the glandular AP content (7). Although this phenomenon has been observed by several authors, it has not been the subject of any experimental studies. We believe that the related decline in intracellular AP concentration may reflect emptying of the parietal cell. Interestingly enough, this reduction in AP content does not occur in mucosal cell preparations, possibly indicating that some paracrine mechanism is involved (Fig. 7).

Summary

We have proposed and provided experimental evidence for the existence of a mechanism controlling the output of hydrochloric acid from the parietal cells. On stimulation of the cells, histamine not only activates the H^+/K-pump but also prevents acid from leaving the cell. The delay may be caused by cAMP activation. When the appropriate conditions have been obtained, the acid leaves the cell and enters the gastric lumen.

A delay in onset of acid secretion is convenient from several points of view. For example, salivary amylase, which is responsible for approximately 50% of the carbohydrate digestion, will have time to exert its digesting effect in the stomach before the pH decreases and the enzyme becomes inactivated. Also, bicarbonate secretion will be started before high concentrations of acid have been obtained in the stomach and thereby contributes to protection of gastric mucosa.

Acknowledgments

The authors express their gratitude to Carin Hulth and Margareta Topel for technical assistance, to Sören Johansson for drawing the figures, and to Prof. Stuart Slorach for linguistic examination.

References

1. Nilsson G, Simon J, Yalow RS, Berson SA. Plasma gastrin and gastric acid responses to sham feeding and feeding in dogs. Gastroent 1972; 63:51–59.
2. Sandvik AK, Waldum HL, Kleveland PM, Schulze Sögnen B. Gastrin produces an immediate and dose-dependent histamine release preceding acid secretion in the totally isolated, vascularly perfused rat stomach. Scand J Gastroent 1987; 22:803–808.
3. Berglindh T, Helander HF, Öbrink KJ. Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. Acta Phys Scand 1976; 97:401–414.
4. Berglindh T, ÖBrink KJ. A method for preparing isolated glands from the rabbit gastric mucosa. Acta Physiol Scand 1976; 96:150–159.
5. Romell B, Girma K, Seensalu R, Nilsson G. Effects of H_1-receptor antagonists on ^{14}C-aminopyrine accumulated in gastric cells from rabbit and pigs. To be published.
6. Batzri S, Gardner JD. Action of histamine on cyclic AMP in guinea pig gastric cells: inhibition by H_1 and H_2-receptor antagonists. Mol Pharmacol 1979; 16:406–416.
7. Norberg L, Ljungström M, Vega FV, Mårdh S. Stimulation of acid formation by histamine, carbachol and pentagastrin in isolated pig parietal cells. Acta Physiol Scand 1986; 126:385–390.

Rosalyn S. Yalow with a guinea pig used for immunization.

The Brain-Gut Axis: A Model System to Study Gene Regulation

R. J. Greenstein, M.D.

It is a pleasure to offer this contribution in celebration of Rosalyn S. Yalow, Solomon A. Berson Professor at Large, whose germinative contributions have so advanced knowledge in the biological sciences in the second half of the twentieth century. I present an overview of the utility of the brain-gut axis in studying differential control of gene expression (1–3). Data in the rat encompassing ontogeny, aging, and nutritional manipulations are assessed.

In the rat, the difference in cell lineages between brain and intestine is most graphically illustrated by cell turnover kinetics. A neuronal cell is produced in the fetal or early neonatal period and is not thereafter replaced during the life cycle of that organism. By contrast, rat intestinal mucosa has the most rapid turnover of any cell line in its body (4). Cell replication takes approximately 10 hours (5). From production at the base of the crypts of Lieberkuhn to being sloughed at the tip of a villus takes 72 hours (6). Constituent elements of the gastrointestinal tract (4) include columnar epithelium (7) and enteroendocrine cells (8).

Cholecystokinin (CCK) provides an elegant marker within the brain-gut axis (Table 1) (1, 9, 10). Other peptides, such as VIP, are considered brain-gut peptides (9). However, they function as a neurotransmitter in both brain and the muscle layer of the intestine. In contrast to VIP, intestinal CCK colocalizes to the mucosa, where it is found in the enteroendocrine cells.

Intestinal CCK has two classically described hormonal actions: gallbladder contraction (11) and protein-rich exocrine pancreatic digestive enzyme secretion (12). In addition to its pancreatic growth factor activity (13), intestinal CCK may function as an intestinal growth factor (14). As a neuropeptide, CCK (15–17) is found throughout the central nervous system. Molecular species and concentrations of CCK differ in the cortex, hypothalamus (18–20), basal ganglia (21), and cerebellum (22). It may be a satiety factor (23–26), a modulator of pain responses (27), and an inhibitory neurotransmitter (28). Despite these observations, the neuronal functions of CCK remain to be fully defined (29).

Understanding of the molecular biology of CCK can be dated to the cloning of a rat CCK cDNA in 1984 (30). The CCK gene has since been cloned in pig (31) and humans (32). Already elucidated is the localization in brain of CCK mRNA (18), differential expression as analyzed by in situ hybridization in vertebrate brain (29), and the ontogenic changes (1) associated with CCK mRNA. Translational work has addressed the molecular species of the resultant CCK peptides (33, 34) and the possible mechanisms of production of these post-translationally modified peptides.

Brain-gut differences have been observed in the rat. Various stages in the biosynthetic pathway have been investigated, from gene through transcription, translation, and post-translational processing.

Methods

Transcriptional Assay. To quantify CCK messenger RNA (CCK mRNA), a solution hybridization assay is used (2). To generate an "antisense" RNAprobe, CCK cDNA is subcloned into a commercially purchased vector (pGEM 3,

---

Supported in part by the Veterans Affairs Medical Center.

From the Laboratory of Molecular Surgical Research, Dept. of Surgery, Veterans Affairs Medical Center, Bronx, NY and Mount Sinai School of Medicine, New York City. Address correspondence and reprint requests to the author at Laboratory of Molecular Surgical Research, VAMC Bronx, 130 W. Kingsbridge Rd., Bronx, NY 10468.

TABLE 1
Rat Brain-Gut Differences, Gene Expression by Tissue Lineage

		Intestine	
	Brain	Muscle	Mucosa
VIP	Epiderm	Epiderm	NF
CCK	Epiderm	?	Endoderm
Secretin	NF	NF	Endoderm

NF, not found.

TABLE 2
Rat Brain-Gut Differences, Ontogeny: Maximal Concentration by Age (Weeks)

	Brain	Gut
CCK		
mRNA*	3	Birth
Large peptides†	4	2
Small peptides†	4	2
VIP†	4	4

* Friedman et al. PNAS 1985;82:5593–5597.

† Ichihara et al. Biochem Biophys Res Comm 1983;112:891–898.

TABLE 3
Rat Brain-Gut Differences, Concentration with Increasing Age

	Brain	Duodenum
Total RNA	Decrease	NC
Total protein	NC	Decrease
CCK large forms	NC	Decrease
CCK$_8$	Decrease	NC
VIP	NC	NC
Secretin	NA	NC

NC, no change; significance, $p \leq 0.05$.
NA, not applicable.

TABLE 4
Rat Brain-Gut Differences during Nutritional Deprivation

	Brain	Gut
Total RNA (content)	NC	Decrease
CCK mRNA (concentration)	NC	NC
CCK mRNA (content)	NC	Decrease
CCK peptides		
Large forms (concentration)	NC	Decrease
Large forms (content)	NC	Decrease
Small forms (conc/cont)	NC	NC
VIP peptides	NC	NC

NC, no change; significance at $p \leq 0.05$.

Promega, Madison, WI). To produce "sense" CCK mRNA, in vitro, the procedure is modified: the plasmid is linearized with the restriction endonuclease cutting at the other end of the insert. The RNA polymerase start site at the other end of the inserted CCK cDNA (SP 6) is then used to incorporate four cold nucleotides (2). An antisense radiolabelled CCK RNA probe is hybridized to either duodenal or brain total RNA (35).

Radioimmunoassay: Translational and Post-Translational Events. The CCK, VIP, and secretin peptide analyses are performed using well-characterized methods in routine use in the Solomon A. Berson Research Laboratory (3, 9, 36–38, 39). The extraction process, methanol followed by aqueous acid, separates the large from the small, post-translationally modified, molecular species of CCK.

Results

Growth (ontogeny), development (aging), and physiologic (nutritional deprivation) differences are reviewed. Results are presented as summaries of published data (Tables 2–4).

Ontogenic studies show a consistent greater delay in the production of both CCK mRNA and peptides in the brain than in the intestine (Table 2) (1, 3). By contrast, there is no such variation with VIP.

Developmental studies of aging show similar differences between the brain and the intestine (10). Decreases in brain RNA (10, 40, 41) are not paralleled in the gut.

In physiologic studies of nutritional deprivation, homeostasis is remarkable in the brain of markedly catabolic animals. There is a profound decrease in the values of CCK peptides observed in the intestine (2, 3), but no variation in the values of VIP.

Discussion

Ontogeny. These observations show that in the neonatal rat the intestine matures at an earlier age than does the brain. This is a reflection of need; presumably in the neonatal rat processing nutrients is more important than advanced brain function. VIP is considered a brain-gut peptide, although it is neuronal in both brain and gut. With VIP, no ontogenic brain-gut difference is observed. This reinforces the utility of studying CCK. Its location in tissues of completely different lineages (epidermal and endodermal) may well account for the differences.

Development. Aging (Table 3) produces decreases in brain RNA (10, 40, 41) not paralleled in the gut. Intestinal stability with increasing age may reflect the ongoing metabolic demands associated with eating. How homeostasis is maintained in the brain despite falling RNA remains to be determined.

Nutrition. In the brain of catabolic animals there is homeostasis (Table 4) (2, 3). There is a profound decrease in the values observed for intestinal CCK. Intestinal VIP, by contrast, does not vary, again reinforcing the utility of studying CCK in the brain-gut paradigm. These data elegantly demonstrate preferential neuronal conservation over the intestine in the nutritionally deprived animal. The mechanisms of regulation remain to be determined.

Summary

The data summarized show unequivocal differences in gene regulation between the brain and the intestine in the rat. It is possible that the differences may occur partly as a reflection of cell turnover kinetics. The mechanisms accounting for these differences should provide fertile ground for investigation. I conclude that the brain-gut axis provides a model for studying differential gene regulation in animals.

References

1. Friedman J, Schneider BS, Powell D. Differential expression of the mouse cholecystokinin gene during brain and gut development. Proc Natl Acad Sci USA 1985; 82:5593–5597.
2. Greenstein RJ, Isola L, Gordon J. Differential cholecystokinin gene expression in brain and gut of the fasted rat. Am J Med Sci 1990; 299:32–37.
3. Zheng B, Eng J, Yalow RS. Brain/gut peptides in fed and fasted rats. Endocrinology 1987; 120:714–717.
4. Wright N, Alison M. Biology of epithelial cell populations. Oxford: Clarendon Press, 1984:551–595.
5. Ferriera MN, Leblond CP. Argentaffin and other endocrine cells of the small intestine in the mouse. 1: ultrastructure and classification. Am J Anat 1971; 131:331–342.
6. Cairnie AB, Bentley J, Lamerton LF, Steel GG. Cell proliferation studies in the intestinal epithelium of the rat. 1: determination of kinetic parameters. Exp Cell Res 1965; 39:528–538.
7. Cairnie AB. Renewal of goblet and Paneth cells in the small intestine. Cell Tissue Kinet 1970; 3:35–45.
8. Ferriera MN, Leblond CP. Argentaffin and other endocrine cells of the small intestine in the adult mouse. 2: Renewal. Am J Anat 1971; 131:331–342.
9. Ichihara K, Eng J, Yalow RS. Ontogeny of immunoreactive CCK, VIP and secretin in rat brain and gut. Biochem Biophys Res Commun 1983; 112:891–898.
10. Greenstein RJ, Ybanez MM, Zhang R-L, Bauman WA. Is aging preprogrammed? Observations from the brain/gut axis. Mech Ageing Dev 1991; 61:113–121.
11. Ivy AC, Oldberg E. A hormone mechanism for gallbladder contraction and evacuation. Am J Physiol 1928; 86:599–613.
12. Harper AA, Raper HS. Pancreozymin: a stimulant of secretion of pancreatic enzymes in extracts of the small intestine. J Physiol (Lond) 1943; 102:115–125.
13. Wood JG, Dale WE, Solomon TE. Effects of chronic administration of CCK on pancreas of the dog and rat. Gastroenterology 1984; 86:1302.
14. Greenstein RJ, Colucci RD, Ybanez MM, Zhang R-L, McElhinney AJ, Bauman WA. Cholecystokinin upregulation during intestinal repair. J Surg Res 1992 (in press).
15. Vanderhaegen JJ, Signeau JC, Gepts W. New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature 1975; 257:604–605.
16. Dockray GJ. Immunochemical evidence of cholecystokinin-like peptides in brain. Nature 1976; 264:568–570.
17. Muller JE, Straus E, Yalow RS. Cholecystokinin and its COOH-terminal octapeptide in the pig brain. Proc Natl Acad Sci USA 1977; 74:3035–3037.
18. Hasegawa M, Usui H, Araki K, Kuwano R, Takahashi Y. Developmental and regional changes of cholecystokinin mRNA in rat brains. FEBS Lett 1986; 194:224–226.
19. Siegel RE, Young WS. Detection of preprocholecystokinin and preproenkephalin A mRNAs in rat brain by hybridization histochemistry using complementary RNA probes. Neuropeptides 1985; 6:573–580.
20. Miceli MO, Van Der Kooy D, Post CA, et al. Differential distributions of cholecystokinin in hamster and rat forebrain. Brain Res 1987; 402:318–330.
21. Jenner P, Taquet H, Mauborgne A, et al. Lack of change in basal ganglia neuropeptide content following subacute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment of the common marmoset. J Neurochem 1986; 47:1548–1551.
22. Gubler U, Chua AO, Young D, Fan Z-W, Eng J. Cholecystokinin mRNA in porcine cerebellum. J Biol Chem 1987; 262:15242–15245.
23. Smith GP. The therapeutic potential of cholecystokinin. Int J Obes 1984; 8:51–63.
24. Wager-Srdar SA, Gannon M, Levine AS. The effect of cholecystokinin on food intake in gonadectomized and intact rats: the influence of sex hormones. Physiol Behav 1987; 40:25–28.
25. Collins SM, Conover KL, Forsyth PA, Weingarten HP. Endogenous cholecystokinin and intestinal satiety. Am J Physiol 1985; 18(6):R667–R671.
26. Schick RR, Yaksh TL, Go VL. An intragastric meal releases the putative satiety factor cholecystokinin from hypothalamic neurons in cats. Brain Res 1986; 370: 349–353.
27. Miceli MO. Role of gastric distention in cholecystokinin's satiety effect in golden hamsters. Physiol Behav 1985; 35:945–953.
28. Sugaya K, Matsuda I, Kubota K. Possible involvement of the CCK receptor in the benzodiazepine antagonism to CCK in the mouse brain. Jpn J Pharmacol 1987; 43:67–71.
29. Goldman SA, Monahan JW, Schneider BS. The regional and subcellular development of cholecystokinin immunoreactivity in vertebrate brain. Brain Res 1985; 22:237–246.
30. Deschenes RJ, Lorenz LJ, Haun RS, Roos BA, Collier KJ, Dixon J. Cloning and sequence analysis of a cDNA encoding rat preprocholecystokinin. Proc Natl Acad Sci USA 1984; 81:726–730.

31. Gubler U, Chua AO, Hoffman BJ, Collier KJ, Eng J. Cloned cDNA to cholecystokinin mRNA predicts an identical preprocholecystokinin in pig brain and gut. Proc Natl Acad Sci USA 1984; 81:4307–4310.
32. Takahashi Y, Kato K, Hayashizaki Y, et al. Molecular cloning of the human cholecystokinin gene by use of a synthetic probe containing deoxyinosine. Proc Natl Acad Sci USA 1985; 82:1931–1985.
33. Rehfeld JF. Accumulation of nonamidated preprogastrin and preprocholecystokinin products in porcine pituitary corticotrophs: evidence of post-translational control of cell differentiation. J Biol Chem 1986; 261:5841–5847.
34. Rehfeld JF, Hansen HF. Characterization of preprocholecystokinin products in the porcine cerebral cortex: evidence of different processing pathways. J Biol Chem 1986; 261:5832–5840.
35. Auffray C, Rougeon F. Purification of mouse immunoglobin heavy-chain messenger-RNA's from total myeloma tumor RNA. Eur J Biochem 1980; 170:303–314.
36. Ichihara K, Eng J, Yalow RS. Ontogeny of molecular forms of CCK-peptides in rat brain and gut. Life Sci 1984; 34:93–98.
37. Ichihara K, Eng J, Pond WG, Yen JT, Straus E, Yalow RS. Ontogeny of immunoreactive CCK and VIP in pig brain and gut. Peptides 1984; 5:623–626.
38. Greenstein RJ, Huang C, McElhinney AJ. Small forms of cholecystokinin are increased in repairing small intestine. Surg Res Commun 1989; 5:141–147.
39. Huang CG, Eng J, Yalow RS. Ontogeny of immunoreactive cholecystokinin, vasoactive intestinal polypeptide, and secretin in guinea pig brain and gut. Endocrinology 1986; 118:1096–1101.
40. Morrison MR, Pardue S, Griffin WS. Altered expression of different tubulin electrophoretic variants during human cortex development. J Neurogenet 1983; 1:105–111.
41. Griffin WS, Morrison MR. In-situ hybridization—visualization and quantitation of genetic expression in mammalian brain. Peptides 1985; 6:89–96.

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society

Mitochondrial Dysfunction

Luft's Disease Revisited

Severe Hypermetabolism of Nonthyroid Origin with a Defect in the Maintenance of Mitochondrial Respiratory Control

Rolf Luft, M.D.

I shall briefly describe the first mitochondrial disorder ever studied and how we have interpreted the findings of further investigations over the years. The first study opened the field of mitochondrial pathophysiology, which has expanded greatly during the last decade (1, 2).

Case Report

This story goes back to 1958, when the patient first came to the attention of my department. She was an unmarried woman, born in 1928, who since 1935 had suffered considerably from enormous perspiration, polydipsia without polyuria, increased appetite, weight loss, and asthenia.

The patient had been well until 1935—she was then 7 years old—when it was noted that her perspiration was progressively increasing; she soon had to change all her clothes up to 10 times a day. She had to drink more and more, approximately 2.5–3 L per day, while her urine volumes remained small. Her appetite also increased markedly, and her caloric intake finally reached 12600–14700 J (3,000–3,500 cal) per day and remained at that level. In spite of this, her body weight became stable at about 39 kg at a body height of 159 cm. The only other abnormal finding at physical examination was a pulse rate at rest of 90–110, and absent knee jerks and Achilles tendon reflexes.

The central laboratory finding was a basal metabolic rate of +180%, which would have been almost twice as high had oxygen consumption been calculated per unit of lean body mass.

The obvious question to be asked at the time was: were the patient's symptoms due to exceptional overproduction of thyroid hormone?

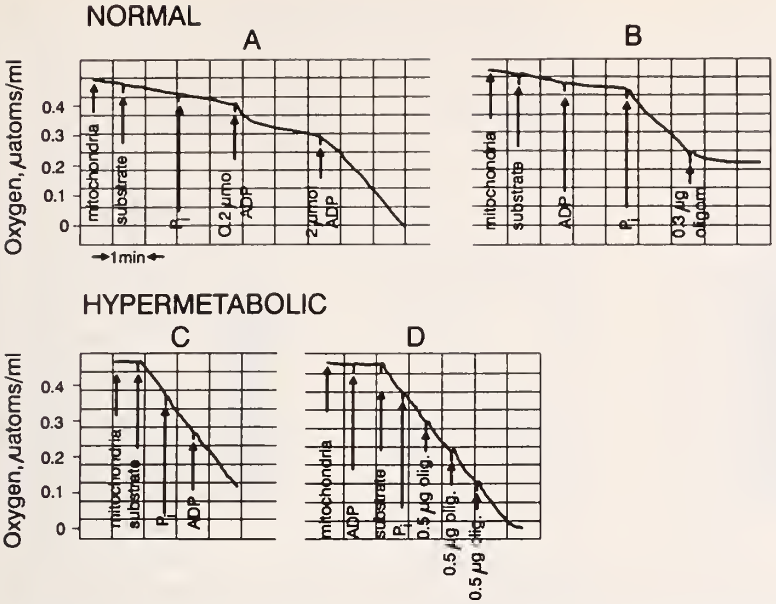
Over the years, the patient's thyroid function had been examined in most of the many hospitals in which she had stayed, and with every possible available technique. At one point, a subtotal thyroidectomy had been performed, followed by treatment with large doses of thiouracil. The result was a myxedematous state with serum cholesterol about 600 mg/100 mL, somewhat decreased perspiration, and a lowering of basal metabolic rate from about +200 to +100%.

After extensive studies of all possible causes of real or indirect oxygen overconsumption, I arrived at a point at which I had to go back to basics and ask the question: What regulates oxygen consumption in the body?

Mitochondria and Respiratory Control. After some consideration, I arrived at the conclusion that the cause of the patient's enormous oxygen consumption had to be sought among those factors which determine the rate of respiration of the resting organism or, in other words, in the energy-transforming mechanism of the cell itself. At this time, it had been clearly established, first, that mitochondria are the site of cell respiration and respiration-linked phosphorylation and, second, that the oxygen uptake of isolated mitochondria can be controlled by the components of adenosine triphosphate (ATP) formation, that is, by inorganic phosphate (P_i) and the phosphate acceptor, adenosine diphosphate (ADP). This is the well-known respiratory control, which forms the

---

From the Department of Endocrinology, Karolinska Institutet, Stockholm, Sweden. Address reprint requests to Professor Emeritus Rolf Luft, Karolinska Institutet, 104 01 Stockholm 60 Sweden.



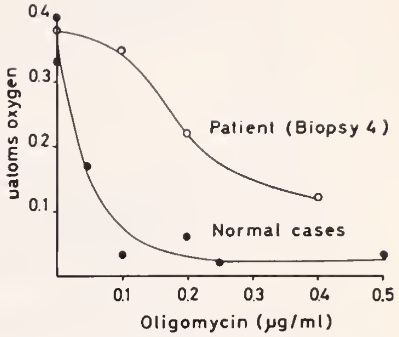


Fig. 1. Left Respiratory control in skeletal muscle mitochondria. NORMAL, subject with normal basal metabolism rate; HYPERMETABOLIC, patient with loose coupling of respiration from phosphorylation. Fig. 2. Right Effect of oligomycin on phosphorylating activity of isolated mitochondria of two healthy subjects and patient with Luft's disease.

basis for the capacity of the body to adapt oxygen consumption to actual energy requirements.

Studies on Mitochondria from Skeletal Muscle. Obviously, we had to study the structure and function of the patient's mitochondria. This turned out to be a major problem. So far, basic studies on mitochondria had only been performed on rat liver mitochondria. Removal of enough liver tissue from the patient to get a mitochondrial pellet that could be used to measure oxidative phosphorylation and respiratory control was not feasible. The only tissue available for this purpose would be skeletal muscle. Also, skeletal muscle seemed appropriate, since the muscle mass of the body is the major determinant of basal metabolic rate. However, at this time, neither mitochondria from rat muscle nor human mitochondria had been studied.

Therefore, a method was developed for isolation of human muscle mitochondria. When this had been completed, the first series of experiments on the patient's muscle mitochondria and those from normal muscle were undertaken.

Our intention was to study the following crucial functions:

1. respiration, phosphorylation, and respiratory control;
2. the effect of oligomycin on respiration and phosphorylation—oligomycin inhibits phosphorylation tightly coupled to respiration;
3. the occurrence of ATP-ase activity and its sensitivity to dinitrophenol.

The mitochondria from the healthy subject (Fig. 1) exhibited a high degree of respiratory control when either phosphate or ATP was lacking. A small amount of ADP in the presence of phosphate caused an instantaneous increase in respiratory rate, followed by a sharp return to the original level when the ADP was exhausted. Addition of 0.3 μg of oligomycin resulted in complete abolition of the respiratory stimulation induced by the presence of phosphate and phosphate acceptor.

The patient's mitochondria began to respire at a high rate as soon as a substrate (in this case pyruvate and malate) was added to the incubation medium, and no further increase occurred on addition of phosphate and ADP. Likewise, when ADP was added prior to the substrate, introduction of the substrate gave rise to a virtually maximal respiratory rate that did not level off with time and could not be influenced by added phosphate. The respiration induced by substrate alone was unaffected by oligomycin, even when this was added in amounts exceeding tenfold that required for maximal inhibition in normal mitochondria.

The phosphorylating activities of the mitochondria are demonstrated in Fig. 2. Glucose in the presence of hexokinase and a catalytic amount of ATP served as terminal phosphate acceptor. The P/O ratio of the normal mitochondria was near 3. Oligomycin, 0.2 μg, again almost completely abolished the effect of phosphate plus phosphate acceptor on respiration.

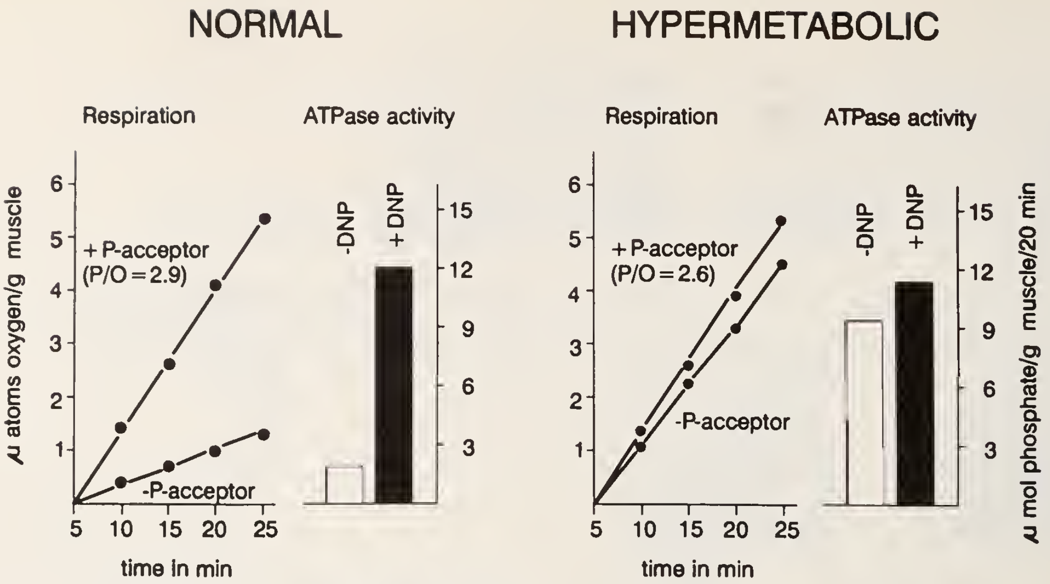


Fig. 3. Respiratory control, phosphorylative efficiency, and ATPase activity of isolated mitochondria of healthy subject (NORMAL) and patient with Luft's disease (HYPERMETABOLIC).

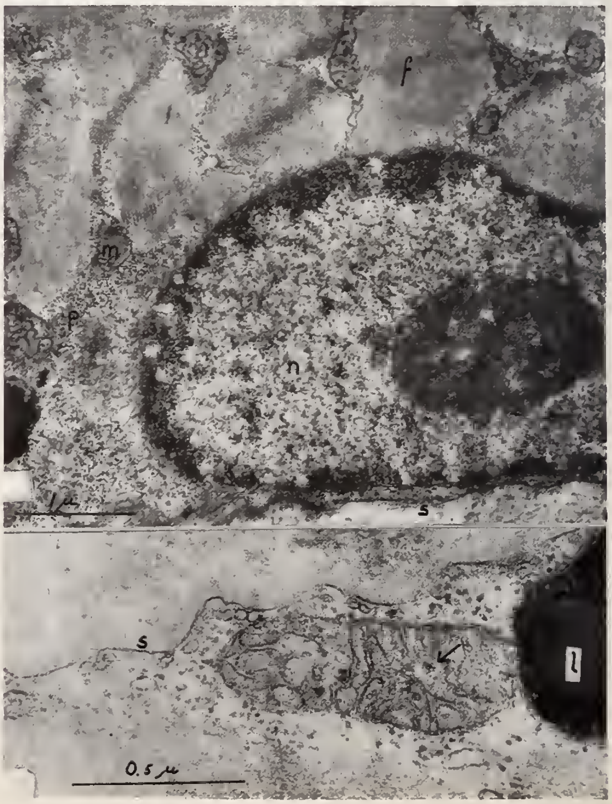
The hypermetabolic patient's mitochondria, in the presence of phosphate and ADP, showed noticeable phosphorylation, with a P/O ratio of 1.57. Oligomycin up to 1 μg suppressed phosphate uptake by more than 90% (P/O ratio 0.2).

To summarize: the finding of absent respiratory control and, to a certain degree, intact phosphorylation in the presence of P and ADP is in accord with the concept of loose coupling of oxidative phosphorylation. Absence of respiratory inhibition by oligomycin, which only inhibits phosphorylation coupled to respiration, also accords with the concept of loose coupling. In addition in the normal mitochondria, ATPase activity was low and markedly stimulated by dinitrophenol (Fig. 3). In contrast, the patient's mitochondria demonstrated high ATPase activity, which was only slightly stimulated by dinitrophenol.

These results gave occasion to the following attending question: Could the supernatant fraction of the patient's muscle contain any endogenous uncoupling factor? No effect of the supernatant fraction from the patient's muscle homogenate was noted on respiratory control of normal mitochondria, using the oligomycin sensitivity of respiration of these mitochondria as a test. The next question was: Is there in the mitochondria of the patient a morphologic substrate to the deranged function?

Morphologic Studies. Electron microscopy of the patient's muscle revealed drastic changes from normal in the morphologic organization of the mitochondria. Electron micrographs of a muscle fiber from a healthy subject (Fig. 4) revealed

Fig. 4. Electron micrograph of thin section through peripheral portion of muscle fiber from healthy subject (top). Myofibrils (f) are tightly packed; only a few mitochondria (m) can be seen. Nucleus (n) with its dense nucleolus is seen close to sarcolemma (s). Portion of same muscle at higher magnification (bottom) showing details of morphology of mitochondrion: outer membrane; inner membranes or christae; to right, a so-called lipofuscin body (l).



tightly packed myofibrils and few mitochondria between these. The normal mitochondrion was surrounded by an outer membrane and contained many inner membranes or christae. In the hypermetabolic patient (Fig. 5, 6), the cell nucleus was surrounded by a thick layer of mitochondria extremely varied in size and shape. A bundle of dense cell inclusions could be seen. In a giant mitochondrion the densely packed christae were arranged in a zigzag. A great variation of other structural changes of the mitochondria were also present.

At this time, we interpreted our findings as expressions of an enormous turnover of mitochondria in the patient's muscle cells. The question we now asked was: Can such increased activity be substantiated by biochemical findings?

Complementary Analysis of Mitochondrial Function. Complementary analysis revealed that the patient's muscle contained 3–4 times more mitochondrial protein than did muscle from control subjects. Furthermore, the cytochrome oxidase activity per unit mitochondrial protein was also higher than in controls, especially in the fraction containing the large mitochondria. Otherwise, there were no differences in the contents of respiratory chain components between the two populations of mitochondria, including co-enzyme Q.

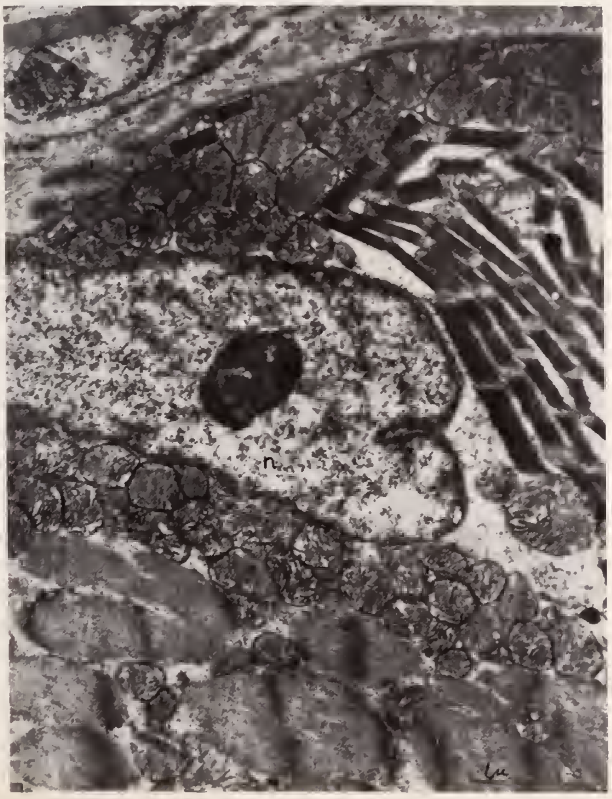
Summary of Findings on Mitochondria. The main findings concerning the muscle mitochondria of this patient were as follows:

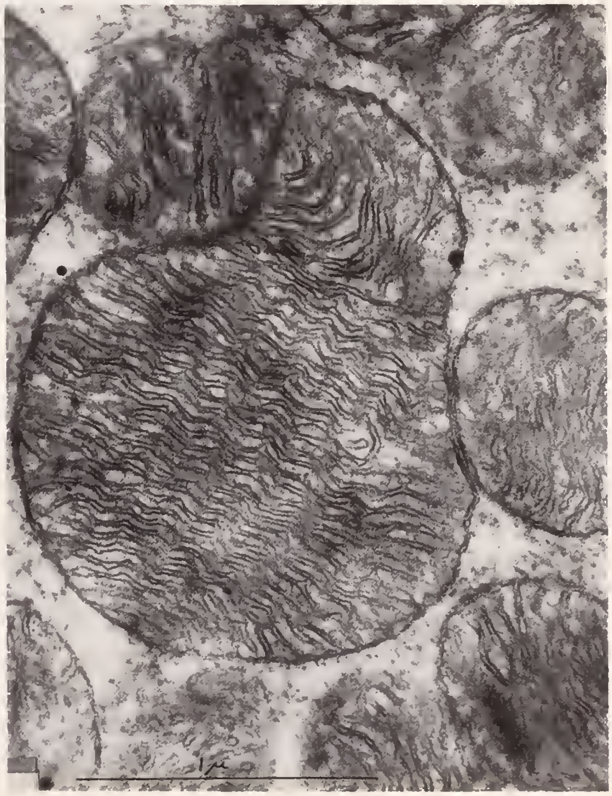
- enormous increase in the number of mitochondria in skeletal muscle
- enormous variation and increase in the size of the mitochondria, indicating increased production and turnover of mitochondria
- aggregation of mitochondria particularly striking in the nuclear area
- increase in size of the nucleoli, indicating high RNA content
- increased content of RNA in the muscle homogenate also on chemical determination
- increased content of respiratory enzymes (for example, cytochrome oxidase) but not of coenzyme Q in the mitochondria

The next question to be asked was: Is there a relationship between the altered respiratory control and the symptoms of the patient?

Relationship of Altered Respiratory Control and Clinical Symptoms. The high respiration of the mitochondria even in the absence of phosphate and ADP explains why the patient was unable to adapt her oxygen consumption to the actual need for energy and, therefore, that metabolism proceeded at a maximal rate contin-

Fig. 5. (Left) Electron micrograph from muscle fiber from patient with Luft's disease: cell nucleus (n) with very dense nucleolus; multitude of mitochondria (m) surrounding nucleus; below this mitochondrial zone, myofibrils with some mitochondria intermingled. Right, bundle of dense cell inclusions of unknown character. **Fig. 6. (Right)** Mitochondria from mitochondrial zone of the patient with Luft's disease: great variation in size and shape; zigzag arrangement of densely packed christae.





uously for 24 hours a day, even during rest. It was probably essential that the mitochondria, in spite of their defective capacity for respiratory control, could still produce ATP—that is, that respiration was loosely coupled to phosphorylation, not uncoupled. Most likely, a complete uncoupling of respiration from phosphorylation would be incompatible with life.

The enormous hypermetabolism explains the main clinical symptoms of the patient:

- increased perspiration, to relieve the body of excess heat
- thirst, to compensate for loss of fluid
- polyphagia, to compensate for the increased combustion of food
- thinness, a sign that caloric intake, although very high, was still only sufficient to sustain very low body weight

Whatever the cause of the syndrome, the patient described here represented the first clinical example of a functional defect of mitochondria, and illustrated dramatically the role of mitochondrial respiratory control in the energy metabolism of the living organism.

Now to the ultimate question: What was the basic cause of the loose coupling of the skeletal muscle mitochondria in this patient?

Pathophysiology of the Disease

I shall summarize my views of the pathophysiology of the disease as they have changed over the years.

Tight coupling of respiration and phosphorylation requires—in addition to an assembly of active enzymes—proper arrangement of these enzymes within the mitochondrial structure. It seemed possible that such accessory structural factors could be in a state of malfunction when mitochondrial respiratory control was impaired, as it was in our patient. A possibility considered at this time was that the patient's mitochondria were lacking a component involved in the maintenance of the watertight environment around the energy coupling sites. If the formation of a component with the tightening function did not keep pace with the overall rate of synthesis of mitochondrial material, the result would be enhanced synthesis of mitochondrial material.

Evidence supporting this proposal came from the increased levels of mitochondrial protein and of cytochrome oxidase activity, as well as the high RNA content of the patient's muscle.

Proton Gradient. A new possibility in approaching the problem of pathophysiology was provided by Peter Mitchell's chemoosmotic theory for oxidative phosphorylation (Nobel prize 1980). According to Mitchell, the basis for the maintenance of respiratory control is a protein gradient over the inner membrane of the mitochondria. If this gradient cannot be maintained, loose coupling or uncoupling—defective energy transport from respiration to phosphorylation—ensues.

One explanation of the patient's condition could then be some kind of damage to the mitochondrial membranes, allowing protons to pass through. This, in turn, would eliminate the proton gradient.

An Altered Protein. Another possibility is that the patient's mitochondria might have contained an altered protein engaged in the translocation of protons and other ions such as calcium. Such a protein might have promoted the proton-calcium transport and, thereby, removed the proton gradient. As a matter of fact, in the second patient described with this disease (1976) (3), considerable loss of calcium ions over the mitochondrial membranes was demonstrated, suggesting an energy-dissipating futile cycle of Ca^{2+} uptake and release.

Perhaps the most attractive hypothesis appreciates the so-called uncoupling protein, discovered some years ago in mitochondria from brown fat, which is abundant in hibernating animals. Normally, the function of this protein is to make the proton gradient collapse in the mitochondria in this fat tissue and, thereby, stimulate the release of respiratory energy as heat. In tissues other than brown fat, this protein is blocked by nucleotides or is not synthesized. We may speculate that this particular protein was present and active in the patient's muscle mitochondria and was responsible for the loose coupling and the ensuing increased production of heat.

Possibilities exist to test all these hypotheses the day a third patient with this disease is discovered.

Coenzyme Q. Most recently, another explanation has been provided for this disease. It goes back to our findings of the occurrence of vast paracrystalline inclusions in the mitochondria, possibly consisting of lipofusein granules, and the occurrence of normal levels of coenzyme Q. In other instances of increased mitochondrial population and respiratory activity of skeletal muscle, the coenzyme Q level is not normal but rather is high. This enzyme, in its reduced form, occupies a central role as a powerful antioxidant, inhibiting lipid peroxidation and, thereby, preventing membrane, protein, and DNA damage and the formation of lipofuscin aggregates.

Coenzyme Q might also be the component involved in the tight coupling of phosphorylation to oxidation. Therefore, the fact that the concentration of coenzyme Q was not properly elevated in Luft's disease may have special significance. Future research in this field will have to deal with the principles of coenzyme Q biosynthesis.

Summary and Outlook

The first description of a mitochondrial disease was published in 1959–62. This opened the field of mitochondrial pathophysiology. In 1976, a second case of this disease was reported. Another breakthrough in the field was the discovery that mitochondria contain 2–10 copies of a small double-stranded circular DNA molecule which is exclusively maternally transmitted. This was followed by findings that major deletions and point mutations of mitochondrial DNA cause human diseases, chiefly mitochondrial myopathies and encephalopathies. The number of reports on mitochondrial diseases has now mounted to several hundred (4). A recent classification of mitochondrial defects by Scholte (1988) contains more than 120 entries (5).

Thus, we have been witnessing the emergence of a new medical field, the scope of which cannot as yet be foreseen. But it all started 30 years ago with studies on a patient at the Department of Endocrinology at the Karolinska Hospital and the Wenner-Gren Institute in Stockholm.

References

1. Ernster L, Ikkos D, Luft R. Enzymic activities of human skeletal muscle mitochondria: a tool in clinical metabolic research. Nature 1959; 184:1851–1854.
2. Luft R, Ikkos D, Palmieri G, Ernster L, Afzelius B. A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical and morphological study. J Clin Invest 1962; 41:1776–1804.
3. DiMauro S, Bonilla E, Lee CP, Schotland DL, Scarpa A, Conn H, Chance B. Luft's disease: further biochemical and ultrastructural studies of skeletal muscle in the second case. J Neurol Sci 1976; 27:217–232.
4. Ernster L, Lee CP. Thirty years of mitochondrial pathophysiology: from Luft's disease to oxygen toxicity. In: Kim CH, Ozawa T, eds. Bioenergetics: molecular biology, biochemistry and pathology. New York: Plenum, 1990, 451–465.
5. Scholte HR. The biochemical basis of mitochondrial diseases. J Bioenerg Biomembr 1988; 20:161–191.

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society

Novel Peptides and Enzymes

Exendin Peptides

John Eng, M.D.

The concept that a chemical assay for carboxyl-terminal amidation could be used to detect and isolate new biologically active peptides was introduced by Tatemoto and Mutt in 1978 (1). Their chemical assay led to the isolation of a series of new amidated peptides from intestine, brain, and pancreatic tissues. These peptides include PHI (2), PYY (3), NPY (4), galanin (5), and pancreastatin (6).

A corollary to their work is that other post-translational peptide modifications may also be used to screen for new peptides with potential biological activity. One frequent biochemical feature of biologically active peptides in the brain-gut axis is the presence of amino-terminal histidine residues (His^1) in peptides that activate intracellular adenylyl cyclase. These His^1 peptides appear to be generated by site-specific cleavages of their respective precursor peptides to yield mature peptides that have an amino-terminal histidine coupled with a phenylalanine at the six position (His^{1}Phe^6).

Historically, glucagon was the prototype His^{1}Phe^6 peptide (7). It was later followed by the isolation and structural identifications of secretin (8) and VIP (9). The number of His^1 peptides has grown since then. More recently, it has been found that preproglucagon may be further processed to produce two other His^{1}Phe^6 peptides, GLP-1 (7–36) and GLP-2, in certain tissues (10). These peptides have been grouped under the glucagon superfamily of peptides (11) (Table), which now encompasses a large collection of peptides that are biologically diverse in their activities but are structurally related by having an amino-terminal histidine and a phenylalanine at position 6 (His^{1}Phe^6) or variant structures such as Tyr^{1}Phe^6 (12, 13), His^{1}Tyr^6 (14) or His^{1}Leu^6 (15).

Assay for His^1. We assayed for His^1 by performing a single cycle of amino acid sequencing, followed by identification of the derivatized amino-terminal residue, and used this chemical assay to monitor the purification of glucagon from chinchilla pancreas after finding that the pancreatic extract had little or no immunoreactivity with a number of glucagon antisera. When chinchilla glucagon was purified and sequenced, it was found to differ from the usual mammalian glucagon by three amino acid substitutions which did not affect its biological activity (16). The purification of chinchilla glucagon with the His^1 assay validated the use of this assay for monitoring the purification of other His^1 peptides.

Isolation of Helospectin, Helodermin, and Exendin Peptides. We then used the His^1 assay to analyze venom from the lizard *Heloderma horridum.* Venom from the closely related lizard *H. suspectum* had originally been reported to stimulate pancreatic enzyme secretion in vitro (17). The peptides helospectin I and II (18) were subsequently isolated from *H. suspectum* venom and found to have amino-terminal histidines in their peptide sequences. Venom from *H. suspectum* was also shown to contain an 86% homologous peptide, helodermin (19), which has pancreatic secretagogue activity and an amino-terminal histidine. Both helospectin and helodermin are His^{1}Phe^6 peptides. When we analyzed venom from *H. horridum* with the His^1 assay, two His^1 peptides were identified, helospectin and a new, previously unrecognized peptide which we named exendin-3.

Exendin-3 Peptide

Structural characterization of exendin-3 by amino acid sequencing and mass spectrometry showed that it is a 39 amino acid peptide having

---

Research supported by U.S. Department of Veterans Affairs.

From the Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, 130 West Kingsbridge Rd., Bronx, NY 10468, and Department of Medicine, Mount Sinai School of Medicine, New York, NY. Address reprint requests to the author at the Berson Research Laboratory.

TABLE

Exendin-3	HSDGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS#
Exendin-4	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS#
Glucagon	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Secretin	HSDGTFTSELSRLRDSARLQRLLQGLV#
VIP	HSDAVFTDNYTRLRKQMAVKKYLNSILN#
GLP-1 (7–36)	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR#
GLP-2	HAEGSFSDEMNTILDNLAARDFINWLIQTKITDR

# carboxyl-terminal amide.

One-letter notation for amino acids: A = Ala, D = Asp, E = Glu, F = Phe, G = Gly, H = His, I = Ile, K = Lys, L = Leu, M = Met, N = Asn, P = Pro, Q = Gln, R = Arg, S = Ser, T = Thr, V = Val, W = Trp, Y = Tyr.

an amino-terminal histidine, a phenylalanine at position 6, and a serine amide at the carboxyl-terminus (20). Exendin-3 is thus the third His^{1}Phe^6 peptide to be isolated from *Heloderma* venom but has only 32% and 26% homologies with helospectin and helodermin, respectively.

Exendin-3 was tested for its effects on cellular cAMP and amylase release in dispersed acini prepared from guinea pig pancreas. It was shown to stimulate the release of amylase but has only 0.01% of VIP's potency as a secretagogue (21). When tested for its effect on cellular cAMP, a biphasic dose-response curve was obtained. At low concentrations (0.1–3 nM), exendin-3 stimulates up to a twelvefold increase in cAMP, whereas at higher concentrations (0.3–3 μM) it stimulates up to an additional 24-fold increase (21). The second phase increase in cellular cAMP stimulated by high concentrations of exendin-3 (>100 nM) correlated with amylase release, suggesting that both were due to interaction with high-affinity VIP receptors.

This interaction was verified by two approaches. Exendin-3 was shown to inhibit the binding of radiolabeled VIP to pancreatic acini over the same range of concentrations required to stimulate amylase release. Secondly, the VIP-receptor antagonists [Ac-Tyr^1,D-Phe^2]GRF (1-29)amide and [4-Cl-D-Phe^6,Leu^{17}]VIP inhibited amylase release by VIP and exendin-3 in an equally efficacious manner. Thus at high concentrations (>100 nM), exendin-3 interacts with high-affinity VIP receptors to produce an increase in cellular cAMP that stimulates amylase release from pancreatic acini.

The first phase increase in cellular cAMP which is stimulated by lower concentrations of exendin-3 (0.1–3 nM) has not yet been correlated with a biological function. Studies have shown that this cAMP response is not due to occupation of secretin-preferring receptors on the acini since exendin-3 does not perturb secretin-stimulated increases in cellular cAMP as it would be expected to do if there were competition between the two peptides for occupancy of those receptors (21). Evidence that this first phase increase in cellular cAMP results from the stimulation of a previously undescribed exendin receptor is provided by the carboxyl-terminal fragment of exendin-3, exendin-3(9-39) amide. This fragment by itself does not affect basal cellular cAMP or amylase release. However, at increasing concentrations it progressively abolishes the first phase increase in cellular cAMP caused by exendin-3 and thus acts as a competitive inhibitor of exendin-3 (21). The inhibitory effect of exendin-3(9-39) amide is specific for exendin-3 and does not affect the actions of VIP, secretin or other agents known to increase pancreatic acinar cAMP.

Exendin-4 Peptide

Additional evidence for the putative exendin receptor comes from studies performed with exendin-4, an analog of exendin-3 that was purified from H. suspectum venom using the His^1 assay (22). We detected and then isolated exendin-4 and helodermin from *H. suspectum* venom but did not find any evidence of helospectin in the venom despite its expected presence (18). A likely explanation is that the venom used in the original purification of helospectin (18) was obtained not from *H. suspectum* but rather from a subspecies of *Heloderma* lizard more closely related to *H. horridum.*

Exendin-4 differs from exendin-3 by two amino acid substitutions at positions 2 and 3 from the amino-terminus. Its amino-terminal sequence is His-Gly-Glu . . . but the peptide is otherwise identical to exendin-3. The biological activity of exendin-4 differs from that of exendin-3. Exendin-4 does not stimulate amylase release from pancreatic acini at concentrations up to 1 μM. In parallel with its apparent inability to interact with the high-affinity VIP receptors, exendin-4 produces only a monophasic increase in cellular cAMP where the second phase increase in cAMP associated with amylase release is absent. The monophasic response of cellular cAMP to exendin-4 stimulation is abolished by exendin(9-39) amide at concentrations greater than 100 nM. Thus, exendin-4 appears to interact solely with the exendin receptor on pancreatic acinar cells.

Summary

Exendin-3 and exendin-4 are biologically active peptides isolated from venoms of the Gila monster lizards, *H. horridum* and *H. suspectum*, respectively. They were isolated using a chemical assay which detects peptides with amino-terminal histidine residues. Both are 39 amino acid peptides containing an amino-terminal histidine and a carboxyl-terminal serine amide and are members of the glucagon superfamily of peptide hormones. When tested in a dispersed pancreatic acinar cell assay, exendin-3 stimulates amylase release and with increasing concentrations causes a biphasic increase in cellular cAMP. In contrast, exendin-4 at concentrations up to 1 μM does not stimulate amylase release and produces a monophasic increase in cellular cAMP despite differing from exendin-3 by only two amino acid substitutions at positions 2 and 3 from the N-terminus.

Endogenous Mammalian Analog to Exendins? The differences in biological activities can be explained by the observation that exendin-3 interacts with VIP receptors to stimulate amylase release, whereas exendin-4 does not. Both exendin-3 and exendin-4 interact with a putative exendin receptor on pancreatic acinar cells. The presence of this receptor was determined and defined by the ability of a specific inhibitor, exendin(9-39) amide, to abolish the increase in cAMP observed with 0.1–3 nM exendin-3 or exendin-4. The presence of the exendin receptor, although functionally undefined at the present time, predicts the existence of an endogenous mammalian analog to the exendin peptides.

References

1. Tatemoto K, Mutt V. Chemical determination of polypeptide hormones. Proc Natl Acad Sci USA 1978; 75:4115–4119.
2. Tatemoto K, Mutt V. Isolation and characterization of the intestinal peptide porcine-PHI (PHI-27): a new member of the glucagon-secretin family. Proc Natl Acad Sci USA 1981; 78:6603–6607.
3. Tatemoto K. Isolation and characterization of peptide YY (PYY): a candidate gut hormone that inhibits pancreatic exocrine secretion. Proc Natl Acad Sci USA 1982; 79:2514–2518.
4. Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y: a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 1982; 296:659–660.
5. Tatemoto K, Rokaeus A, McDonald TJ, Jornvall H, Mutt V. Galanin: a novel biologically active peptide from porcine intestine. FEBS Lett 1983; 164:124–128.
6. Tatemoto K, Efendic S, Mutt V, Makk G, Feistner GF, Barchas JD. Pancreastatin: a novel pancreatic peptide that inhibits insulin secretion. Nature 1986; 324:476–478.
7. Bromer WW, Sinn LG, Behrens OK. The amino acid sequence of glucagon. J Am Chem Soc 1957; 79:2807–2810.
8. Mutt V, Jorpes JE, Magnusson S. Structure of porcine secretin. The amino acid sequence. Eur J Biochem 1970; 15:513–519.
9. Said SI, Mutt V. Isolation from porcine-intestinal wall of a vasoactive octacosapeptide related to secretin and glucagon. Eur J Biochem 1972; 28:199–204.
10. Orskov C, Holst JJ, Knuhtsen S, Baldissera FG, Poulsen SS, Nielsen OV. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. Endocrinology 1986; 119:1467–1475.
11. Bell GI. The glucagon superfamily: precursor structure and gene organization. Peptides 1986; 7(Suppl 1):27–36.
12. Jornvall H, Carlquist M, Kwauk S, Otte SC, McIntosh CH, Brown JC, Mutt V. Amino acid sequence and heterogeneity of gastric inhibitory polypeptide (GIP). FEBS Lett 1981; 123:205–210.
13. Guillemin R, Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg W. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science 1982; 218:585–587.
14. Andrews PC, Hawke DH, Lee TD, Legesse K, Noe BD, Shively JE. Isolation and structure of the principal products of preproglucagon processing, including an amidated glucagon-like peptide. J Biol Chem 1986; 261:8128–8133.
15. Gossen D, Bucail L, Cauvin A, Gourlet P, De Neef P, Rathe J, Robberecht P, Vandermeers-Piret M-C, Vandermeers A, Christophe J. Amino acid sequence of VIP, PHI and secretin from the rabbit small intestine. Peptides 1990; 11:123–128.
16. Eng J, Kleinman WA, Chu L-S. Purification of peptide hormones from chinchilla pancreas by chemical assay. Peptides 1990; 11:683–685.
17. Raufman J-P, Jensen RT, Sutliff VE, Pisano JJ, Gardner JD. Actions of Gila monster venom on dispersed acini from guinea pig pancreas. Am J Physiol 1982; 242:G470–G474.
18. Parker DS, Raufman JP, O'Donohue TL, Bledsoe M, Yoshida H, Pisano JJ. Amino acid sequences of helospectins, new members of the glucagon superfamily, found in Gila monster venom. J Biol Chem 1984; 259:11751–11755.
19. Hoshino M, Yanaihara C, Hong YM, et al. Primary structure of helodermin, a VIP-secretin-like peptide. FEBS Lett 1984; 178:233–239.
20. Eng J, Andrews PC, Kleinman WA, Singh L, Raufman JP. Purification and structure of exendin-3, a new pancreatic secretagogue isolated from *Heloderma horridum* venom. J Biol Chem 1990; 265:20259–20262.
21. Raufman JP, Singh L, Eng J. Exendin-3, a novel peptide from *Heloderma horridum* venom, interacts with vasoactive intestinal peptide receptors and a newly described receptor on dispersed acini from guinea pig pancreas. J Biol Chem 1991; 266:2897–2902.
22. Eng J, Kleinman W, Singh L, Raufman J-P. Isolation of exendin-4, an analog of exendin-3, from *Heloderma suspectum* venom. Digestion 1990; 46(suppl 1):29.

Identification of a Novel Reductase in Folate Metabolism

Sheldon P. Rothenberg, M.D., David Kyner, Ph.D., and Gabriel Solomon, M.A.

Abstract

A protein with a molecular weight of 27,500 was co-purified with the enzyme dihydrofolate reductase (molecular weight 22,000) from the liver of mice less than 8 weeks of age using a methotrexate-Sepharose affinity matrix. This 27.5 kDa protein crossreacts with dihydrofolate reductase against an antiserum raised to the purified 22 kDa enzyme. The protein could also reduce dihydrofolate to tetrahydrofolate, thus demonstrating the catalytic properties of dihydrofolate reductase. The expression of this 27.5 kDa protein also appears to be age-dependent because it could not be isolated from liver of mice older than four months.

My tenure (SPR) in the Berson-Yalow lab was the singular event in my early postdoctoral training that launched me on the academic career I pursue today. I was fortunate to be in their laboratory as the development of the radioimmunoassay for insulin (1) reached the scientific community, and I have witnessed, as has everyone else—including the Nobel Prize Selection Committee—the impact of this discovery on all of biomedical science. Although my research interest has focused on various aspects of vitamin B_{12} and folate metabolism, the identification of a novel reductase (2) which is the subject of this report resulted from prior observations that were made by the application of radioimmunoassay to study nonfunctional variants of the enzyme dihydrofolate reductase (E.C. 1.5.1.3.) (3).

Folic acid is an essential vitamin in both eukaryotic and prokaryotic cellular metabolism. Folate in the reduced form, as tetrahydrofolate, serves as a carrier of carbon units for a number of metabolic pathways, including the synthesis of purines and thymidine, the methylation of homocysteine for the synthesis of methionine, the interconversion of serine and glycine, and the metabolism of histidine (4). In this role as a cofactor for most of these enzymatic pathways, the tetrahydrofolate form of folate is conserved. However, in the process of the methylation of deoxyuridine monophosphate for the de novo synthesis of thymidine monophosphate, the folate cofactor, N^5N^{10} methylene tetrahydrofolate, is oxidized to dihydrofolate (FH_2). FH_2 is then reduced to tetrahydrofolate by an NADPH-dependent reduction that is catalyzed by dihydrofolate reductase (5).

Our initial studies of this enzyme were concerned with its activity in human tissues because, unlike the enzyme in animal tissues, it has been difficult to measure in most human tissues, both normal and neoplastic (6). Accordingly, to distinguish between enzyme activity and enzyme protein we developed, naturally, a radioimmunoassay for dihydrofolate reductase using an antiserum we raised to this enzyme purified from L1210 murine leukemia cells (3). This antiserum also reacted by immunoprecipitation with a protein in human leukemia cells, even though these cells did not contain catalytically active dihydrofolate reductase (7).

An Unexpected Finding. To characterize this immunoreactive protein, we subjected the cytosol from human leukemia cells to gel filtration chro-

---

Supported by a research grant from the Department of Veterans Affairs Research and Development Service and the Kresevich Foundation of New York.

From the Division of Hematology/Oncology, Department of Medicine, Department of Veterans Affairs Medical Center and State University Health Science Center, Brooklyn, NY. Address reprint requests to Sheldon P. Rothenberg, MD, 800 Poly Place, Brooklyn, NY 11209.

matography and assayed each fraction for immunoreactive protein (3). The interesting and unexpected finding was that this crossreacting protein eluted from the column before active dihydrofolate reductase. Thus, this nonfunctional immunoreactive protein appeared by gel filtration to have a greater molecular weight than the active enzyme. This finding initially suggested to us that perhaps this enzyme is synthesized in human tissues as an inactive zymogen which is then activated by cleavage of a peptide to yield the active enzyme which has a molecular weight between 20,000 and 22,000 (8).

Animal Tissues. At this point in our studies, we began to study animal tissues for two reasons. First, these tissues are readily available for such study and second, they contain more active dihydrofolate reductase than most human tissues.

We began with the mouse of the BDF_1 strain as the source of tissue because the antiserum to dihydrofolate reductase that we used for our initial studies was raised against the enzyme purified from L1210 leukemia cells (3), a mouse murine leukemia that we carried in this species.

For this initial study we followed a rather simple procedure (9). Cytosol was prepared from three readily accessible tissues—liver, kidney, and brain. We chose these tissues because the liver and kidney are known to contain active dihydrofolate reductase, whereas the brain contains little or no active enzyme (10). The cytosol prepared from these tissues was subjected to gel-filtration chromatography through Sephadex G-75, as we did with the human leukemia cell cytosol (3), and immunoreactive protein was then determined in each fraction by radioimmunoassay. Functional dihydrofolate reductase was also determined in each fraction by measuring the catalytic reduction of folate to tetrahydrofolate (11) and by the binding of ^{3}H-methotrexate (12).

Another Unexpected Finding. The results of these studies were unexpected and most interesting (9). The liver cytosol contained two peaks of immunoreactive protein, one at the void volume of the column and one eluting in the included column volume with an apparent molecular weight of 20,000–22,000. Functional dihydrofolate reductase activity was found only with the smaller molecular weight immunoreactive protein.

The cytosol of the kidney, in contrast, contained very little high-molecular-weight immunoreactive protein which eluted in the void volume of the column, and virtually all of the immunoreactive and functional activity eluted in the fractions containing active dihydrofolate reductase. Thus, the kidney differed from the liver by containing essentially only active enzyme.

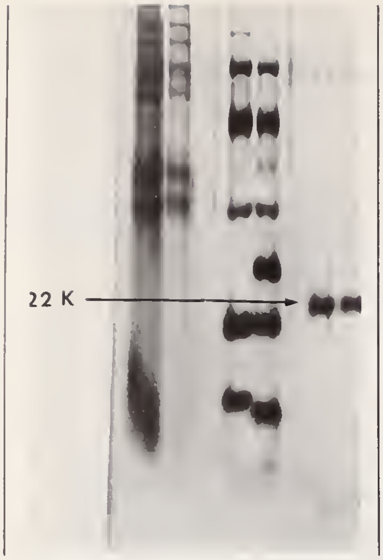
A surprising finding was made with a similar analysis of cytosol from mouse brain. All of the immunoreactive protein eluted in the high molecular weight void volume of the column and there was neither immunoreactive protein nor functional enzyme activity eluting in the position of active dihydrofolate reductase.

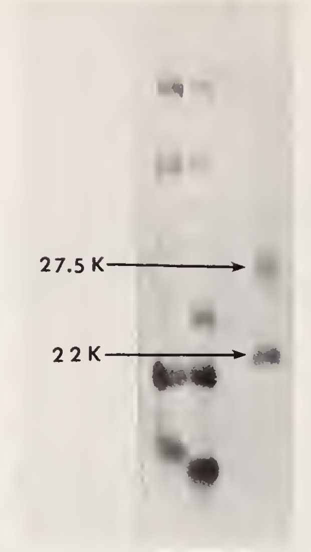
But the identification of this high-molecular-weight crossreacting protein that appears to be some homologue of dihydrofolate reductase was not the stopping-point; it was only an introduction to the studies that led to the identification of the novel reductase reported recently in detail (2).

Serendipity. As so often occurs in scientific research, this protein was discovered by serendipity. After completing the series of experiments I just described, we had nearly exhausted our supply of antiserum to dihydrofolate reductase and proceeded to purify additional enzyme for purposes of immunization. However, rather than using L1210 leukemia cells as the source of dihydrofolate reductase (which requires harvesting a great many cells from many leukemic mice), we found it easier to purify the enzyme from a pool of livers from normal mice. We used essentially the same procedure that we previously described (7) for the purification of the enzyme from calf liver. After homogenization of the tissue and partial purification by salt precipitation, the enzyme is isolated by affinity chromatography through a methotrexate-Sepharose matrix (7). Figure 1 shows the SDS-PAGE of dihydrofolate reductase purified by this method from the liver of mice. Only a single protein was obtained; it had a molecular weight of 22,000, which is the same as that of dihydrofolate reductase. The apparent molecular weight of the enzyme is 22,000.

When we exhausted this preparation of purified dihydrofolate reductase, additional enzyme was prepared by the identical procedure. This time, however, the final preparation obtained from the methotrexate affinity matrix contained two proteins, one with an apparent molecular weight of 22,000 (dihydrofolate reductase) and a second with an apparent molecular weight of 27,500.

We initially assumed from this finding that this preparation contained a contaminating protein, so we reapplied it to a fresh methotrexate affinity column, washed it extensively again, and then eluted the enzyme in the customary way, using FH_2 to displace the enzyme (7). To our surprise, we found the two bands shown in Fig. 2. A





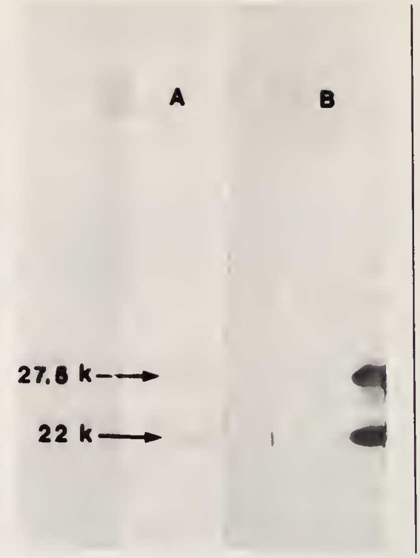


Fig. 1. **Left** SDS-PAGE of dihydrofolate reductase purified from liver of mice. Arrow, lanes containing purified enzyme which has a molecular weight of 22,000. Preceding four lanes contain the molecular weight marker proteins. Fig. 2. **Middle** SDS-PAGE of dihydrofolate reductase purified from liver of mice by same method used for preparation in Fig. 1. Arrows, enzyme which has a molecular weight of 22,000, and a second protein which has a molecular weight of 27,500. Fig. 3. **Right** SDS-PAGE and immunoblot of preparation of dihydrofolate reductase purified from liver of young mice which also contains the 27,500 molecular-weight protein. After electrophoresis, proteins were transferred to an Immobilon membrane. Strip A was exposed to normal rabbit serum, Strip B to an antiserum raised in a rabbit against the 22,000 molecular-weight dihydrofolate reductase purified from L1210 murine leukemia cells. Half of strip B was removed for protein staining.

Western blot of this SDS-PAGE separation of the two proteins (Fig. 3) showed that both reacted with the antiserum raised against purified dihydrofolate reductase (molecular weight 22,000) from L1210 leukemia cells.

Some Clues. In order to resolve this puzzling observation we proceeded to purify yet another batch of dihydrofolate reductase. This time, however, we obtained the results we expected the first time, just the 22 kDa dihydrofolate reductase. To explain this perplexing observation, we reviewed in detail the steps we followed for each preparation and found only one difference between the two. For the preparation that yielded both the 22 kDa and 27.5 kDa proteins, the mice had just been delivered to the laboratory and were, therefore, young, and the mean weight of the livers was 1.4 g. In contrast, the mean weight of the livers used for the preparation that yielded only the 22 kDa enzyme was 2.4 g, because the mice by this time had grown older.

This was the first clue that could explain our findings—that the expression of the 27.5 kDa protein was limited to the liver of young mice. This hypothesis proved to be correct, because both 22 kDa enzyme and the 27.5 kDa protein were obtained again when we purified dihydrofolate reductase from the liver of both young mice and newborn mice, but only the 22 kDa enzyme was obtained from the liver of mice older than 5 months (2).

What I have described until now is a protein with an apparent molecular weight of 27,500 which co-purifies with dihydrofolate reductase when the liver of young mice is the source of the enzyme. The only evidence suggesting that this protein is functionally similar to dihydrofolate reductase is that it binds to methotrexate in the purification procedure. However, that is only a ligand-binding property and not a catalytic property. The protein, for example, could be a folate-binding protein which may also bind methotrexate (13).

Searching for Catalytic Activity. To determine whether this protein has catalytic activity proved to be a formidable task because we could only separate the two proteins using denaturing SDS gel electrophoresis. All other methods that we attempted for the separation of these proteins without denaturation, such as ion exchange, gel filtration, and hydrophobic interaction chromatography, proved unsuccessful (2). Finally, we used a nondenaturing gel electrophoresis system by which we could determine catalytic activity for the reduction of dihydrofolate with a gel-overlay method described by Mell and co-workers (14). With a 5%–12.5% gradient polyacrylamide gel (non-SDS), we could separate three proteins in the purified preparation from the livers of young mice.

The color is generated by reduction of MTT-tetrazolium and in this instance the reducing agent is the tetrahydrofolate generated by the NADPH-dependent reduction of dihydrofolate by functional dihydrofolate reductase. In control experiments in which either the substrate, or NADPH, or the purified preparation was omitted from the overlay gel, the color reaction was not observed.

Having established that this preparation from the liver of young mice contains three catalytically active proteins, we still did not know which enzyme corresponded to the 27.5 kDa protein. To determine this, we eluted separately each protein corresponding to the chromogenic position on the gel and subjected each eluate to SDS-PAGE.

The slowest moving of the three chromogenic bands proved to correspond to the protein with an apparent molecular weight of 22,000, while the fastest moving chromogenic band had an apparent molecular weight of 27,500. The middle of the three chromogenic bands appeared to contain both the 22 kDa and 27.5 kDa proteins.

Thus, we established that this 27.5 kDa protein, which was purified from the liver of young mice, not only binds to the methotrexate ligand but also has catalytic activity for the reduction of dihydrofolate to tetrahydrofolate (2).

Further Characterization. At this point in the study we have turned to molecular genetics to further characterize this protein. With the help of John Eng, we were able to obtain an amino acid sequence from a cyanogen bromide generated fragment of the 27.5 kDa protein, and we have prepared a corresponding oligonucleotide mixture to screen a cDNA library which had been prepared from the liver of young mice. We have isolated and purified four clones from this library, and we are now in the process of determining the nucleotide sequence of the longest insert. This will provide the amino acid sequence of the protein and indicate the nature of its homology to the 22 kDa dihydrofolate reductase which has also been cloned from the mouse and well characterized (15).

I (SPR) again acknowledge the importance of my tenure in the Berson-Yalow laboratory for launching me on my career in biomedical research. I want also to thank Ros for always being available to provide advice as I encountered puzzling problems during the course of my work.

References

1. Yalow RS, Berson SA. Immunologic specificity of human insulin: application to immunoassay of insulin. J Clin Invest 1961; 40:2190–2198.
2. Kyner D, Rothenberg SP. Age dependent expression of a novel protein in mouse liver immunologically and functionally homologous with dihydrofolate reductase. Biochim Biophys Acta 1989; 993:56–62.
3. Rothenberg SP, Iqbal MP. Human leukemia and normal leukocytes contain a species of immunoreactive but nonfunctional dihydrofolate reductase. Proc Natl Acad Sci USA 1982; 79:645–649.
4. MacKenzie RE. Biogenesis and interconversion of substituted tetrahydrofolates. In: Blakley RL, Benkoric SJ, eds. Folates and pterins, vol. I. John Wiley & Sons, 1984:255–306.
5. Blakely RL. The biochemistry of folic acid and 12 related pteridines. Amsterdam: North Holland, 1969:139–187.
6. Whitehead WM, Kamen BA, Beaulieu D. Levels of dihydrofolate reductase in livers of birds, animals, primates and man. Drug Del 1987; 4:185–189.
7. Iqbal MP, Rothenberg SP. A nonfunctional protein in human leukemia cells reacts with antiserum to dihydrofolate reductase from L1210 leukemia cells. Life Sci 1981; 29:689–696.
8. Albrecht AM, Biedler JL, Hutchinson DR. Two different species of dihydrofolate reductase in mammalian cells differentially resistant to amethopterin and methasquin. Cancer Res 1972; 23:1539–1546.
9. Iqbal MP, Rothenberg SP, LaRosa C. Mouse brain contains a high molecular weight nonfunctional protein with antigenic homology to dihydrofolate reductase. Biochem Biophys Res Commun 1986; 141:258–262.
10. Makulu OR, Smith EF, Bertino JR. Lack of dihydrofolate reductase activity in brain tissue of mammalian species: possible implications. J Neurochem 1973; 21:241–245.
11. Rothenberg SP. A rapid radioassay for folic acid reductase and amethopterin. Anal Biochem 1966; 16:176–179.
12. Rothenberg SP, da Costa M, Iqbal MP. Ligand-binding radioassay for the antifolate compounds: application in patients receiving methotrexate (MTX). Cancer Treat Rep 1977; 61:575–584.
13. Kane MA, Portillo RM, Elwood PC, Antony AC, Kolhouse JF. The influence of extracellular folate concentrations on methotrexate uptake by human KB cells. J Biol Chem 1986; 261:44–49.
14. Mell GP, Martelli M, Kirschner J, Huennekens FM. Multiple forms of dihydrofolate reductase. Biochem Biophys Res Commun 1968; 33:74–79.
15. Stone D, Paterson SJ, Raper JH, Philips AN. The amino acid sequence of dihydrofolate reductase from the mouse lymphoma L1210 cells. J Biol Chem 1979; 254:480–488.

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society

Biosynthesis and Metabolism

Albumin Metabolism:

A Brief Review

Marcus A. Rothschild, M.D., and Murray Oratz, Ph.D.

Serum albumin is the major protein circulating in plasma. The level of serum albumin is determined by the relationship of three processes: synthesis, degradation, and distribution.

In 1839 H. Ancell wrote, "Albumin is doubtless one of the most important of the animal proximate principles. It is found not only in the serum of the blood but in the lymph, chyle, in the exhalations from surfaces, in the fluid of cellular tissue in the aqueous and vitreous humors of the eye, in many other animal fluids. Albumin acts sometimes as an acid and a base and its powers of maintaining its combinations is so great." He also noted that albumin proportions are "pretty nearly the source in all higher animals, the levels are nearly the same in the sexes and between the ages of 20–60."

It is hard to imagine a more comprehensive and succinct definition of albumin 152 years later.

Albumin Synthesis. Albumin synthesis and albumin degradation are not interrelated. The amount of albumin produced per day averages about 200 mg/kg of body weight per day, or about 15 g in the average adult. It was this stability, plus the fact that hyperalbuminemia is not seen except in acute dehydration, that suggested that albumin synthesis might be operating at a maximal rate under normal conditions. To test this concept, large doses of thyroid hormone were administered, and albumin synthesis rapidly increased. Similar increases were noted later employing adrenocortical hormones.

The exact intracellular mechanism for these changes has not been elucidated to my knowledge, but clearly one or more of the steps regulating gene expression to RNA synthesis must be involved. Albumin is transported through the liver in a transit time of about 20–30 minutes. There is mandatory secretion of albumin through the Golgi apparatus, and albumin is not stored within the cell. Thus, alteration in this pathway could not explain the marked increases in albumin production seen under the above stimuli.

One other phenomenon is associated with hyperalbuminemia. Kreek made the observation that patients addicted to heroin had elevated albumin levels, even with hepatitis. Albumin synthesis was then found to be elevated in rabbits receiving methadone. Unfortunately, the exact mechanism is still unclear.

Hypoalbuminemia is common, and the level of serum albumin has long been employed as an index of the state of health. Malnutrition and fasting rapidly reduce albumin production, which returns to normal levels once this stress is removed. Other stresses such as infection, trauma, carcinoma, and surgery also result in a decrease in albumin synthesis. These stress-related events are probably mediated by cytokine activity.

The rapidity with which albumin synthesis increases has suggested that albumin in RNA has a long half-life. A protective mechanism for the albumin in RNA has been described in the cytosol. Thus it may be rapidly available for albumin synthesis.

Other factors which alter or lower albumin production are toxins such as alcohol and CCl_4. Acute exposure to alcohol at levels found in our drinking population has been shown to reduce albumin synthesis in the perfused rabbit liver. Certain amino acids, but not all, added in excess to

---

From the Nuclear Medicine Service, Veterans Affairs Medical Center, New York, NY; The University of Miami Medical Center, and New York University College of Dentistry, New York, NY. Address reprint requests to Marcus A. Rothschild, M.D., at 21770 Cypress Drive, No. 21D, Boca Raton, FL 33433.

the perfusate can prevent this decrease in albumin synthesis. One of these amino acids is ornithine, which is not incorporated into protein. Since ornithine and arginine are precursors of the polyamines, studies were conducted with arginine and with the polyamine spermine. Both were found to be effective in restoring albumin production and were synergistic. The acute effects of alcohol perfusion could be reversed or prevented.

One of the major functions of serum albumin is to maintain plasma colloid osmotic pressure. Clinically, hypergammaglobulinemia is associated with hypoalbuminemia. The infusion of dextran or gamma globulin or the hyperimmunization of rabbits results in a rapid and significant decrease in albumin synthesis. This finding has recently been demonstrated at the gene level. The infusion of excess serum albumin does not result in a significant change in albumin production. The accumulation of excess plasma colloid is prevented by a marked increase in albumin degradation, as well as urinary loss.

Albumin Degradation. Albumin degradation remains mainly a mystery. We do know that the rate of albumin degradation is mass dependent, depending on the serum albumin level. As the level drops, fractional degradative rate falls, as in nephrosis. Albumin synthesis does not necessarily increase in this condition. The site of degradation is not known, and it appears that old and newly synthesized albumin molecules are degraded at the same rate. Albumin survives with a half-life of about 3 weeks in the plasma of humans. The turnover rate is faster in small animals, however.

Albumin Distribution. Albumin is widely distributed throughout the body, the extracellular compartments accounting for over 60% of the total exchangeable albumin mass. The largest function of this pool of nonplasma albumin is in the skin. The leakage of plasma albumin into extravascular sites averages about 4% per hour, a rate not increased significantly in heart disease or in cirrhosis. Nearly all of the newly synthesized albumin reaches the plasma pool via the lymph. In patients with ascites secondary to portal hypertension, as much as 80% of the newly synthesized albumin bypasses the lymph and enters the ascitic fluid directly. This amount of albumin, plus what is already present in ascites fluid, can account for a significant amount of ascitic fluid accumulation. The ascitic albumin pool, which may be hundreds of grams, exchanges back into the lymph and plasma at a slow rate. The infusion of salt-poor albumin will temporarily increase the serum level, but urinary excretion and increased degradation and redistribution limit the effectiveness of this procedure.

Purposes and Clinical Use. Albumin serves many purposes in the body. The most important are transport and maintaining oncotic pressure. It is amazing that patients with analbuminemia do as well as they do. A great deal more needs to be learned at the molecular level concerning the regulation of synthesis. Why the liver cell alone produces albumin is always interesting. Where and how albumin is degraded is not known, and a change in the albumin molecules preparing them for degradation has not been observed. Many substitutes for albumin as plasma expanders have been tried, but albumin prepared from donor plasma is most frequently employed in countries where it is available. Clearly, greater understanding of the metabolic cycle will assist our intelligent clinical use of albumin.

References

Rothschild MA, Oratz M, Schreiber SS. Serum albumin. Hepatology 1988; 8(2):385–401.

Human Eicosanoid Biosynthesis and Metabolism

Carlo Patrono, M.D., Giovanni Ciabattoni, M.D., and Paola Patrignani, Ph.D.

Eicosanoids are ubiquitous autacoids derived from arachidonate metabolism through various enzyme- or free radical-catalyzed oxidative reactions (1, 2). The resulting biologically active derivatives include prostaglandins (PGs), thromboxane (TX)A_2, and sulfidopeptide leukotrienes (LTs).

These substances are produced at a very low rate, under physiologic circumstances, act in autocrine or paracrine fashion, and are rapidly metabolized in vivo. In the case of prostanoids, the methyl end side chain is first modified, leading to formation of the major circulating metabolites, 15-keto-13,14-dihydro-PGs. Further ω- and β-oxidation leads to chain-shortened products that are excreted in the urine. The majority of prostaglandin and thromboxane metabolites are thus C_{18} or C_{16} compounds, resulting from one or two cycles of β-oxidation. Recent evidence suggests that peroxisomal β-oxidation is of major importance for in vivo degradation of PGs in humans (3).

Identification of major urinary eicosanoid metabolites has led to the development of analytical techniques, such as gas chromatography/mass spectrometry (GC/MS) and radioimmunoassay (RIA). The availability of this methodology for clinical investigation has allowed considerable progress over the last 10 years in our understanding of the pathophysiologic significance of eicosanoid synthesis and action.

In this paper, we intend to review studies carried out in our laboratory using RIA methodology for the assessment of eicosanoid biosynthesis and metabolism in humans.

Prostacyclin Biosynthesis And Metabolism

Although prostacyclin (PGI_2) was initially characterized as a circulating hormone continuously released by the lungs into the arterial circulation, the validity of this concept has been questioned by a number of later findings in healthy subjects. These include (a) comparably low levels of its stable hydrolysis product 6-keto-$PGF_{1\alpha}$ in the pulmonary and brachial arterial blood; (b) very low or undetectable (<7.5 pg/mL) levels of 6-keto-$PGF_{1\alpha}$ in peripheral venous blood; (c) low rate (0.08–0.10 ng/kg · min) of endogenous secretion; (d) rapid disappearance of exogenously infused PGI_2 (reviewed in ref. 4). Since PGI_2 infusion rates of 2.5–5.0 ng/kg · min are required in order to achieve threshold plasma concentrations necessary to alter hemodynamic function or to inhibit platelet function, these findings are consistent with a local rather than systemic nature of PGI_2 action. Extensive enzymatic degradation occurring in the lungs, liver, and kidneys, rather than chemical instability, has been suggested to account for the short-lived presence of PGI_2 in the human circulation (half-life 3 min; ref. 5).

PGI_2 biosynthesis has been described in human renal medullary and cortical microsomes (6), and evidence has been reported that PGI_2 acts as a local modulator in controlling juxtaglomerular function (5). In our study, we found that the intravenous infusion of furosemide, which is known to stimulate renin release via a cyclooxygenase-dependent mechanism, caused a three-to-fourfold increase of urinary 6-keto-$PGF_{1\alpha}$ excretion rate

---

Supported by a grant from Consiglio Nazionale delle Ricerche (Progetto Finalizzato "Prevenzione e controllo dei Fattori di Malattia" Sottoprogetto "Controllo della Patologia Cardiovascolare": 91.00216.PF41).

From the Departments of Pharmacology, University of Chieti School of Medicine, 66013 Chieti; and Catholic University School of Medicine, Largo F. Vito 1, 00168 Rome, Italy. Address reprint requests to Professor Carlo Patrono, Istituto di Farmacologia, Università di Chieti, G. D'Annunzio, via dei Vestini 31, 66013 Chieti, Italy.

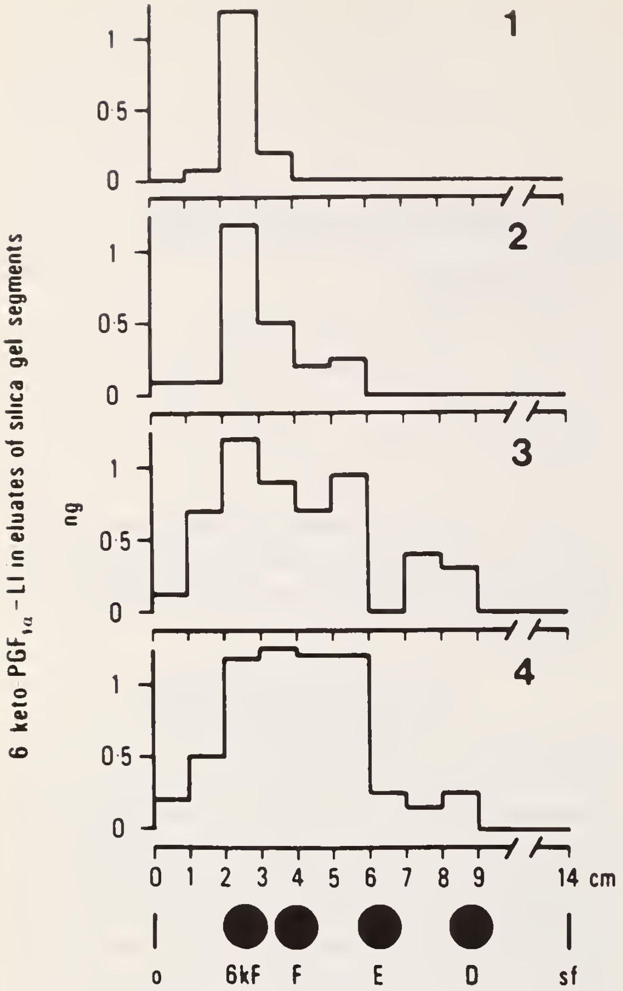


Fig. 1. Heterogeneity of urinary 6-keto-PGF$_{1\alpha}$-like immunoreactivity (LI). Purified extracts, prepared from urine obtained under basal conditions, were subjected to thin-layer chromatography. Whole lane corresponding to one particular extract was divided into 1-cm segments, the silica-gel scraped off and eluted with methanol. All eluates were assayed for 6-keto-PGF$_{1\alpha}$-LI with four anti-6-keto-PGF$_{1\alpha}$ sera of different specificities. Vertical marks on abscissa, origin (o) and solvent front (sf) of plate; dots, location of cochromatographed authentic PGs: 6kF, 6-keto-PGF$_{1\alpha}$; F, PGF$_{2\alpha}$; E, PGE$_2$; D, PGD$_2$. *Reproduced* with permission from ref. 5.

in healthy women, while 6-keto-PGF$_{1\alpha}$ remained undetectable in peripheral venous plasma (5).

The identification of the immunoreactive material present in human urine as 6-keto-PGF$_{1\alpha}$ was obtained by several independent criteria: (a) immunochemical behavior identical to authentic 6-keto-PGF$_{1\alpha}$; (b) chromatographic behavior identical on TLC to authentic 6-keto-PGF$_{1\alpha}$ (Fig. 1); (c) detection by four different anti-6-keto-PGF$_{1\alpha}$ sera; and (d) verification of its identity by GC/MS (5). Thus, separation of immunoreactive species on TLC and their characterization by means of antisera of different specificities allowed us to obtain some sort of immunochromatographic spectrum of the urinary material, along the same line of characterization of the heterogeneity of circulating immunoreactive species of peptide hormones pioneered by Yalow and Berson in the early seventies.

The detection and characterization of 6-keto-PGF$_{1\alpha}$ in human urine led us to suggest its renal origin (5), a suggestion largely supported by subsequent findings (reviewed in ref. 7). Measurement of urinary 6-keto-PGF$_{1\alpha}$ excretion allowed us to demonstrate alterations in renal PGI$_2$ secretion in chronic glomerular disease (8) and lupus nephritis (9) and to characterize selective pharmacologic modulation of its biosynthesis by nonsteroidal antiinflammatory drugs in health (10) and disease (8, 9).

Thromboxane Biosynthesis And Metabolism

TXA$_2$ is a labile eicosanoid affecting contractile elements of the circulating blood (platelets), vasculature (smooth muscle cells), kidney (mesangial cells) and respiratory apparatus (bronchial smooth muscle cells). In platelets, TXA$_2$ represents an amplifying mechanism of activation and aggregation, by virtue of its being synthesized and released in response to a variety of agonists (including collagen, epinephrine, ADP, thrombin) and in turn inducing platelet aggregation (4).

The sequence of nonenzymatic and enzymatic transformations occurring after TXA$_2$ synthesis and release can be depicted as follows:

$$\begin{array}{lcccccc} & & & \text{liver, kidney} & & & \\ & \text{H}_2\text{O} & & \text{lung enzymes} & & & \\ \text{TXA}_2 & \rightarrow & \text{TXB}_2 & \rightarrow & \text{TXM} & \rightarrow & \text{renal clearance} \\ \text{½life:} & 30 & & 5\text{–}7 & & 15\text{–}60 & \\ & \text{sec} & & \text{min} & & \text{min} & \end{array}$$

If we are dealing with the whole human being, rather than with isolated platelets, this sequence of events will inevitably go to completion once triggered by an appropriate pathophysiologic change (such as platelet activation at a site of vascular injury). Our chances of detecting such a sudden (mostly unpredictable) and short-lasting change in TXA$_2$ synthesis and release will depend essentially on three factors: (a) the survival time of the biochemical marker we decide to monitor; (b) the frequency (in the case of plasma) or the duration (in the case of urine) of sampling in relation to the acute event; (c) the possibility of detecting a "signal" that has been generated locally, above a "background noise" represented by the basal rate of total body synthesis of the same substance. The latter also implies that the signal is not artificially amplified by ex vivo platelet activation, as inevitably occurs during and after blood sampling.

It is obvious from the foregoing considerations that measurements of TXB_2 in peripheral venous plasma cannot, under any circumstances, reliably reflect changes in TXA_2 synthesis and release occurring in vivo, because (a) TXB_2 disappears rapidly from the human circulation (11), and (b) it can be "added" to plasma by a minimal degree of platelet activation occurring in relation to blood sampling. As for the measurement of circulating long-lived enzymatic metabolites of TXB_2 (2,3-dinor-TXB_2 and 11-dehydro-TXB_2, for example) that bypass the problem of ex vivo platelet activation, frequent blood sampling around a suspected clinical event would still be required to provide meaningful information.

Potential limitations inherent in the measurement of the same metabolites in urine are related to (a) the cellular source(s) of urinary TXB_2 metabolites; (b) their vascular site(s) of origin; and (c) the timing of metabolite excretion in relation to altered biosynthesis (12).

We have demonstrated a wide discrepancy between the capacity of human platelets to synthesize and release TXA_2 when maximally stimulated ex vivo (for example by endogenous thrombin during whole blood clotting at 37°C) and the actual rate of TXA_2 secretion into the human circulation (11). Thus, the platelets present in 1 mL of human whole blood have the capacity to generate the same amount (300–400 ng) of TXB_2 during 1 hour clotting in vitro as the total body synthesis in vivo over the same period of time. It appears likely that the low biosynthetic rate measured in healthy subjects (0.11 ng/kg · min) reflects the low frequency and/or intensity of stimuli to TXA_2 production (11).

As a consequence, measurements of ex vivo TXB_2 production in whole blood or platelet-rich plasma (PRP) are unlikely to be relevant to changes occurring in vivo, except to reflect drug-induced changes in cyclooxygenase or TX-synthase activity (13).

TXB_2 has two major metabolic pathways in humans (14), one involving β-oxidation resulting in the formation of 2,3-dinor-TXB_2, the other involving dehydrogenation of the hemiacetal alcohol group at C-11, resulting in the formation of a series of metabolites with a delta-lactone ring structure. Although the β-oxidation pathway was originally described as the prevailing form of enzymatic degradation of infused TXB_2 in humans (14), the dehydrogenation pathway has received considerable attention over the last five years following the demonstration that 11-dehydro-TXB_2 has a substantially longer plasma half-life (15) and is excreted at a higher rate (16) than 2,3-dinor-TXB_2.

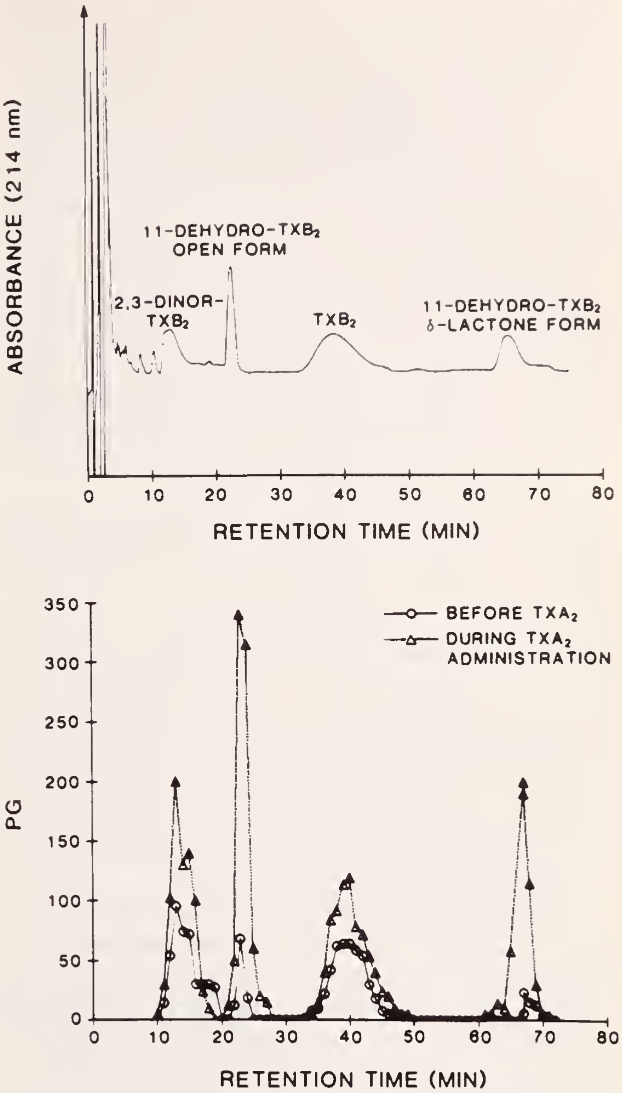


Fig. 2. **Top** Separation of TXB_2, 2,3-dinor-TXB_2 and 11-dehydro-TXB_2 (open and lactone forms) by RP-HPLC. **Bottom** Distribution of TXB_2-, 2,3-dinor-TXB_2-, and 11-dehydro-TXB_2 (open and lactone forms)-like immunoreactivity, as detected by RIA in HPLC fractions of two urine samples obtained before and during TXA_2 infusion. *Reproduced* with permission from ref. 20.

Because the relative contribution of these two pathways of TXB_2 metabolism might vary under pathophysiologic circumstances associated with platelet activation, we have recently characterized the fractional conversion of TXB_2 to urinary 11-dehydro-TXB_2 and 2,3-dinor-TXB_2 over a 50-fold range of infusion rates in healthy volunteers (17). In this study, the fractional elimination of 11-dehydro-TXB_2 was independent of the rate of TXB_2 infusion and averaged 6.8 ± 0.7%. This figure was not significantly different from the corresponding value of 6.4 ± 1.2% for the conversion of TXB_2 to 2,3-dinor-TXB_2 simultaneously measured in the same healthy volunteers (17). Moreover, our estimate of the fractional conversion of systemically administered cold TXB_2 into urinary 11-dehydro-TXB_2 is in agreement with the 7.6% figure obtained following the infusion of radiolabeled TXB_2 at a much higher rate (65 ng/kg · min) (14).

This finding of a relatively constant fractional conversion of TXB_2 to 11-dehydro-TXB_2 over a wide range of entry rates of the parent compound into the circulation (0.1–65 ng/kg · min) should be contrasted with the apparent discrepancy between the fractional elimination of 2,3-dinor-TXB_2 at 0.1–5.0 ng/kg · min—approximately 6% (11)—and that measured at 65 ng/kg · min, which is 23% (14). This might imply that one enzyme system may saturate faster than the other. Thus, measurement of 11-dehydro-TXB_2 might more accurately than 2,3-dinor-TXB_2 reflect changes in TXB_2 production associated with intense and/or long-lasting platelet activation. Measurement of both would obviate the theoretical possibility of drug- and/or disease-dependent alteration in the metabolic disposition of TXB_2. In fact, altered TXB_2 metabolism has been recently shown to occur in association with cigarette smoking (18).

All the studies of thromboxane biosynthesis and metabolism have relied on measurements of TXB_2 metabolites, on the assumption that TXA_2 is hydrolyzed to TXB_2 before being converted into its enzymatic derivatives. However, no information is available on the metabolic fate of TXA_2 in vivo. Because of its chemical instability, natural TXA_2 has not been isolated and characterized as a pure compound. Not until 10 years after discovery of TXA_2 was chemical synthesis of its proposed structure achieved by Bhagwat et al. (19). This has allowed direct comparison of the metabolism of synthetic TXA_2 and TXB_2 in the cynomolgus monkey (20). To measure TXB_2 and its metabolites in urine, we developed a reversed-phase HPLC separation technique, allowing specific recognition of the immunoreactive eicosanoids by RIA (Fig. 2). Similar metabolic disposition of TXA_2 and TXB_2 was demonstrated in this study, thereby suggesting that TXA_2 is likely to hydrolyze to TXB_2 before being converted to 11-dehydro-TXB_2 and 2,3-dinor-TXB_2. Thus, measurement of the urinary excretion of these metabolites provides a reliable indication of in vivo TXA_2 biosynthesis and release.

The availability of these analytical tools has allowed us to demonstrate that TXA_2 biosynthesis is chronically enhanced in association with several cardiovascular risk factors, such as diabetes mellitus (21) and hypercholesterolemia (22). Moreover, increased TXA_2 biosynthesis is episodically expressed in acute coronary (23, 24) and cerebral (25) ischemic syndromes.

TABLE

Differential Pattern of Excretion of TXB_2 and 2,3-Dinor TXB_2 in Health and Disease

	Urinary excretion	
Variable	TXB_2	2,3-Dinor TXB_2
TXB_2 at 0.1 ng/kg · min	no change	time-dependent increase
Aspirin (20–160 mg/day)	no change	dose-dependent decrease
Sulindac (200–800 mg/day)	no change	dose-dependent decrease
Lupus nephritis	2-5–fold increase	no change

Based on data from references 9–11, 26, 27.

The urinary excretion of unmetabolized TXB_2 may largely reflect the intrarenal production of TXA_2 (7). Consistent with this hypothesis, drugs that selectively spare renal cyclooxygenase activity, such as sulindac (10) and low-dose aspirin (26, 27), do not affect urinary TXB_2, although these agents dose-dependently suppress the excretion of the corresponding 2,3-dinor derivative. The differential pattern of excretion of the hydrolysis product of TXA_2 and the corresponding 2,3-dinor metabolite (Table) is consistent with the contention that the pattern of excretion of the hydrolysis products of TXA_2 primarily reflects intrarenal sites of synthesis (such as glomerular epithelial and mesangial cells) (7).

Urinary TXB_2 excretion is increased in patients with lupus nephritis, and the severity of this biochemical alteration is correlated with the activity of renal lesions and with deteriorating renal function (9). These findings prompted us to test the short-term effects of a selective TXA_2-receptor antagonist, BM 13,177, in this clinical setting. The observed improvement of glomerular filtration rate and renal blood flow suggests that in lupus nephritis, impairment of renal function is at least in part mediated hemodynamically and reversible (27). Whether these short-term changes translate into long-term benefits in modifying the progression of lupus nephritis remains to be determined.

Leukotriene C_4 Biosynthesis And Metabolism

The sulfidopeptides LTC_4, LTD_4, and LTE_4 are potent mediators of smooth muscle cell contraction and may be responsible for a wide variety of disorders, including bronchoconstriction in asthma, vasoconstriction in cardiovascular disease, or vasopermeation such as that observed in inflammatory or allergic syndromes (28). The role of these sulfidopeptides in diseases has been suspected mainly because of their pharmacologic properties. Assessment of their production in vivo, in relationship to disease states, should provide insight into their pathophysiologic significance. Both LTE_4 and its ω- and β-oxidation products have been identified in urine, following the infusion of LTC_4 (29) or LTE_4 (30) in healthy subjects. We have recently measured the excretion of LTE_4 during the infusion of exogenous LTC_4 over a 25-fold dose range to enable estimation of the rate of entry of endogenous LTC_4 into the bloodstream of normal humans (Maclouf, Antoine, De Caterina, et al., unpublished data). The fractional elimination of LTE_4 was independent of the rate of LTC_4 infusion and averaged 4.3 ± 0.9%. Interpolation of LTE_4 values obtained under basal conditions onto the linear relationship between the quantities of infused LTC_4 and the amount of LTE_4 excreted in excess of control values permitted calculation of the mean rate of entry of LTC_4 into the circulation as 0.06 pmol/kg · min. Statistically significant changes in the urinary excretion of LTE_4 were measured in association with simulated short-term (10–30 min) increases of LTC_4 entry into the bloodstream. In addition, we characterized further metabolism of LTE_4 by infusing $[^{14}C]LTC_4$ in two of the same subjects. The two major urinary metabolites were the ω- and β-oxidation products identified as the conjugated tetraene 16-COOH-LTE_4 and 14-COOH-LTE_3, which accounted for 6%–8% of the total infused amount of $[^{14}C]LTC_4$. Although the excretion of LTE_4 fell to almost undetectable levels 2h after cessation of the infusion, the presence of the ω/β-oxidized metabolites persisted for up to 15h.

Thus, the present findings suggest that (a) LTC_4 is produced at a low rate under physiologic circumstances and is rapidly converted in the vasculature to LTE_4; (b) changes in the urinary excretion of LTE_4 may reliably reflect short-term changes in the rate of secretion of LTC_4; and (c) measurement of the ω- and β-oxidation products may reflect long-term changes in cysteinyl leukotriene biosynthesis.

Summary and Conclusions

The development of RIA techniques for the measurement of urinary eicosanoid metabolites has allowed characterization of their biosynthesis and metabolism in health and disease. PGI_2, TXA_2, and LTC_4 share a very low rate of secretion into the human circulation and rapidly disappear from circulation because of extensive enzymatic degradation. Long-term changes as well as episodic increases in their biosynthesis can be detected in association with various disease states, as reflected by increased excretion of major enzymatic metabolites such as 2,3-dinor-6-keto-$PGF_{1\alpha}$, 11-dehydro-TXB_2, and LTE_4. Application of the same investigative approach to other recently identified products of arachidonate metabolism (2) may provide further insight into the pathophysiologic significance of this complex mediator system.

Acknowledgments

The expert editorial assistance of G. Protasoni and M. L. Bonanomi is gratefully acknowledged.

References

1. Smith WL, Marnett LJ, De Witt DL. Prostaglandin and thromboxane biosynthesis. Pharmacol Ther 1991; 49:153–179.
2. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts II LJ. A series of prostaglandin F_2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA 1990; 87:9383–9387.
3. Diczfalusy U, Kase BF, Alexson SEH, Björkhem I. Metabolism of prostaglandin $F_{2\alpha}$ in Zellweger syndrome: peroxisomal β-oxidation is of major importance for in vivo degradation of prostaglandins in humans. J Clin Invest 1991; 88:978–984.
4. Oates JA, FitzGerald GA, Branch RA, Jackson EK, Knapp HR, Roberts II LJ. Clinical implications of prostaglandin and thromboxane A_2 formation. N Engl J Med 1988; 319:689–698.
5. Patrono C, Pugliese F, Ciabattoni G, et al. Evidence for a direct stimulatory effect of prostacyclin on renin release in man. J Clin Invest 1982; 69:231–239.
6. Hassid A, Dunn MJ. Microsomal prostaglandin biosynthesis of human kidney. J Biol Chem 1980; 255:2472–2475.
7. Patrono C, Dunn MJ. The clinical significance of inhibition of renal prostaglandin synthesis. Kidney Int 1987; 32:1–12.
8. Ciabattoni G, Cinotti GA, Pierucci A, et al. Effects of sulindac and ibuprofen in patients with chronic glomerular disease: evidence for the dependence of renal function on prostacyclin. N Engl J Med 1984; 310:279–283.
9. Patrono C, Ciabattoni G, Remuzzi G, et al. Functional significance of renal prostacyclin and thromboxane A_2 production in patients with systemic lupus erythematosus. J Clin Invest 1985; 76:1011–1018.
10. Ciabattoni G, Boss AH, Patrignani P, et al. Effects of sulindac on renal and extrarenal eicosanoid synthesis. Clin Pharmacol Ther 1987; 41:380–383.
11. Patrono C, Ciabattoni G, Pugliese F, Pierucci A, Blair IA, FitzGerald GA. Estimated rate of thromboxane secretion into the circulation of normal humans. J Clin Invest 1986; 77:590–594.
12. Patrono C, Ciabattoni G, Patrignani P. Biochemical indices of arachidonate metabolism by platelets and vascular endothelium *in vivo*. In: Patrono C, FitzGerald GA,

eds. Platelets and vascular occlusion. New York: Raven Press, 1989:193–212.

13. Patrono C, Ciabattoni G, Patrignani P, et al. Clinical pharmacology of platelet cyclooxygenase inhibition. Circulation 1985; 72:1177–1184.
14. Roberts II LJ, Sweetman BJ, Oates JA. Metabolism of thromboxane B_2 in man: identification of twenty urinary metabolites. J Biol Chem 1981; 256:8384–8393.
15. Lawson JA, Patrono C, Ciabattoni G, FitzGerald GA. Long-lived enzymatic metabolites of thromboxane B_2 in the human circulation. Anal Biochem 1986; 155:198–205.
16. Catella F, FitzGerald GA. Paired analysis of urinary thromboxane B_2 metabolites in humans. Thromb Res 1987; 47:647–656.
17. Ciabattoni G, Pugliese F, Davì G, Pierucci A, Simonetti BM, Patrono C. Fractional conversion of thromboxane B_2 to urinary 11-dehydrothromboxane B_2 in man. Biochim Biophys Acta 1989; 992:66–70.
18. Uedelhoven WM, Rützel A, Meese CO, Weber PC. Smoking alters thromboxane metabolism in man. Biochim Biophys Acta 1991; 1081:197–201.
19. Bhagwat SS, Hamann PR, Still WL, Bunting S, Fitzpatrick FA. Synthesis and structure of the platelet aggregation factor thromboxane A_2. Nature 1985; 315:511–513.
20. Patrignani P, Morton H, Cirino M, et al. Fractional conversion of thromboxane A_2 and B_2 to urinary 2,3-dinor-thromboxane B_2 and 11-dehydrothromboxane B_2 in the cynomolgus monkey. Biochim Biophys Acta 1989; 992:71–77.
21. Davì G, Catalano I, Averna M, Notarbartolo A, Strano A, Ciabattoni G, Patrono C. Thromboxane biosynthesis and platelet function in type II diabetes mellitus. N Engl J Med 1990; 322:1769–1774.
22. Davì G, Averna M, Catalano I, et al. Increased thromboxane biosynthesis in type IIa hypercholesterolemia. Circulation (in press).
23. Fitzgerald DJ, Roy L, Catella F, FitzGerald GA. Platelet activation in unstable coronary disease. N Engl J Med 1986; 315:983–989.
24. Vejar M, Fragasso G, Hackett D, et al. Dissociation of platelet activation and spontaneous myocardial ischemia in unstable angina. Thromb Haemost 1990; 63:163–168.
25. Koudstaal PJ, Ciabattoni G, vanGijn J, Nieuwenhuis K, de Groot P, Sixma JJ, Patrono C. Thromboxane (TX) biosynthesis in acute cerebral ischemia. Clin Res 1991; 39:146A (abstract).
26. FitzGerald GA, Oates JA, Hawiger J, Maas RL, Roberts II LJ, Lawson JA, Brash AR. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic aspirin administration in man. J Clin Invest 1983; 71:676–688.
27. Pierucci A, Simonetti BM, Pecci G, et al. Improvement of renal function with selective thromboxane antagonism in lupus nephritis. N Engl J Med 1989; 320:421–425.
28. Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway: biochemistry and relation to pathobiology in human diseases. N Engl J Med 1990; 323:645–655.
29. Örning L, Kaijsier L, Hammarström. *In vivo* metabolism of leukotriene C_4 in man: urinary excretion of leukotriene E_4. Biochem Biophys Res Commun 1985; 130:214–220.
30. Sala A, Voelkel N, Maclouf J, Murphy RC. LTE_4 elimination and metabolism in normal human subjects. J Biol Chem 1990; 265:21771–21778.

Author's Note: The work cited on page 161, col. 1, as Maclouf, Antoine, De Caterina, unpublished communication, is now Maclouf J, Antoine C, De Caterina R, et al. Entry rate and metabolism of leukotriene C4 into the vascular compartment in healthy subjects. Am J Physiol (in press). It should also be cited at the end of the sentence just before the head "Summary and Conclusions."

Coronary Artery Disease:

Metabolic Risk Factors and Latent Disease in Individuals with Paraplegia

William A. Bauman, M.D., Ann M. Spungen, M.S., Mohummad Raza, M.D., Jeffrey Rothstein, B.S.,
Run-Lin Zhang, M.D., You-Gong Zhong, M.D., Masako Tsuruta, M.D., Rubina Shahidi, M.D.,
Richard N. Pierson, Jr., M.D., Jack Wang, M.S., and Stanley K. Gordon, M.D.

Abstract

Individuals with spinal cord injury (SCI) currently have a longer life span as a result of recent improvements in medical care. As in the able-bodied population, cardiovascular disease is the leading cause of death in persons with SCI, but it appears to occur at younger ages in those with SCI than in the able-bodied population. The reduction in level of activity and adverse changes in body composition caused by SCI have profound metabolic consequences that may influence the progression and severity of coronary artery disease. Metabolic sequelae of SCI include disorders of carbohydrate and lipid metabolism. Almost half of the 45 active, healthy subjects with paraplegia we studied have a disorder of carbohydrate tolerance, 1 in 5 subjects having a diabetic oral glucose tolerance test. Hyperinsulinemia is found in those with abnormal glucose tolerance. Subjects with paraplegia having impaired glucose tolerance or diabetes mellitus are significantly older than those with normal glucose tolerance. High-density lipoprotein cholesterol is markedly depressed, and low density lipoprotein is relatively elevated. Radionuclide myocardial perfusion imaging after upper body ergometry exercise reveals latent coronary artery disease in 12 of 19 subjects with paraplegia.

Prior to the advent of antibiotics and improvements in genitourinary care, individuals who suffered a spinal cord injury (SCI) generally had a short survival span. In one epidemiologic study (1), in this population genitourinary disorders accounted for 43% of the deaths in the 1940s and 1950s, but only 10% of the deaths in the 1980s and 1990s. Since persons with SCI currently live longer than they once did, diseases of the general aging population will affect the individual with SCI. Thus, cardiovascular disease has replaced genitourinary sepsis as the leading cause of death (1–3). Cardiovascular diseases are the most frequent cause of death among persons surviving more than 30 years after injury (46% of all deaths) and among persons more than 60 years of age (35% of deaths) (1). Cardiovascular disease deaths occur at considerably younger ages in those with SCI than in the able-bodied population.

In this report, we address the metabolic mechanisms which underlie the premature incidence of coronary artery disease (CAD) in persons with SCI and present data on the diagnosis of latent CAD by radionuclide perfusion myocardial imaging in these individuals.

Supported by Solomon A. Berson Research Laboratory; VA Merit Review entitled "Carbohydrate Metabolism in Paraplegics: Potential Benefits of Exercise"; Eastern Paralyzed Veterans Association; and the Veterans Affairs Medical Center, Bronx, NY.

Abstracts presented at AFCR/ASCI/AAP, Seattle, WA, May 1991; American Paraplegic Society, Las Vegas, NV, Sept. 1991.

From the Spinal Cord Damage Research Center and Departments of Medicine and Rehabilitation Medicine, Mount Sinai Medical Center, New York, NY; Spinal Cord Injury and Cardiology Services, and The Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, Bronx, NY; and Body Composition Unit, Columbia/St. Luke's-Roosevelt Hospital Center, New York, NY. Address reprint requests to William A. Bauman, M.D., Director, Spinal Cord Damage Research Center, SCI Service, Room 1E-15, Veterans Affairs Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468.

Subjects

Included in this report are data on 45 healthy veterans in the New York City Metropolitan Area with paralysis due to spinal cord damage, 25 to 70 years of age, who are followed up as outpatients at the Bronx Veterans Affairs Medical Center. Their mean age was 49 ± 2 years; body mass index (BMI), 26.3 ± 0.6 kg/m^2; and duration of injury, 16 ± 2 years. Included in a portion of the study were 57 able-bodied matched control veterans in an outpatient cholesterol screening program whose mean age was 52 ± 1 years and whose BMI was 27.2 ± 0.7 kg/m^2. Informed consent was obtained from all participants. Institutional research and human-studies approvals were obtained for all studies.

Studies and Methods

Study I: Oral Glucose Tolerance. A 3-hour, 75-g oral glucose tolerance test (OGTT) was performed on the 45 male veterans with paraplegia. Subjects were studied in their usual state of health. In the morning after an overnight fast, blood was drawn at −5 and 0 min before and 30, 60, 90, 120, and 180 min after administration of an oral glucose load. Blood for glucose was collected into a test tube containing sodium fluoride, and for insulin, into a test tube containing heparin. Blood was immediately centrifuged and separated for analysis or frozen (−20°C) until analysis was performed. After standardization, determination for glucose was performed on a Glucose II Analyzer (Beckman Instruments). Plasma insulin levels were obtained by radioimmunoassay using methods established in this laboratory (4, 5). The criteria for the diagnosis of normal, impaired glucose tolerance, or diabetes mellitus were those of the National Diabetes Data Group (6).

Study II: Serum Lipids. Fasting lipoprotein determinations were performed on the 45 veterans with paraplegia and the 57 veteran controls. Total cholesterol and triglycerides were assayed on SMAC (Technion Instruments) using the enzymatic method. High-density lipoprotein (HDL) cholesterol was determined by the dextran sulfate method (Technicon HDL cholesterol precipitating reagent, Technicon Instruments Corp., Tarrytown, NY) with enzymatic quantification. Low-density lipoprotein (LDL) cholesterol was calculated from the equation: LDL cholesterol = TC − (HDLc) − (TG/5), where TC is total cholesterol, HDLc is HDL cholesterol, and TG are triglycerides.

Study III: Determinations of Insulin Sensitivity, Cardiopulmonary Fitness, and Body Composition. An intravenous glucose tolerance test, upper-body ergometry metabolic stress test, and body composition studies were performed in a subset of 8 subjects with paraplegia.

An intravenous glucose tolerance with tolbutamide was performed using the modified minimal-model method of Bergman (7). After placement of the two intracatheters, four basal samples were collected over 20 min, after which glucose (0.3 g/kg) was injected over 1 min. An additional injection of tolbutamide (Orinase IV, Upjohn, Kalamazoo, MI) was given over 20 sec, 20 min after glucose. Subjects with BMI <30 kg/m^2 were given 300 mg tolbutamide; subjects with BMI >30 kg/m^2 were given 500 mg. Blood samples were collected at the following times after glucose injection: at t = 0:2, 4, 8, 19, 22, 30, 40, 50, 60, 70, 90, 120, and 180 min. The insulin sensitivity index was calculated from the fast sampling intravenous glucose tolerance (FSIGT) test results, using the program MINMOD, on the VAX-750 at the USC Physiology and Biophysics Department. This program accepts as input the temporal pattern of plasma insulin during the FSIGT, and must fit a simple (minimal) model of insulin-dependent glucose utilization to the measured glucose pattern.

Cardiopulmonary fitness was determined by an upper-body ergometry metabolic stress test. Determination of fitness was accomplished by measurement of oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation (VE), heart rate (HR), and work (watts) (8, 9). A metabolic cart (system 2900, SensorMedics Corp.) was used to measure $\dot{V}O_2$, $\dot{V}CO_2$, and VE. A heart rate telemeter (Polar Pacer, Computer Instruments Corp.) was used to measure HR. Work was measured by use of a Fleisch Universal Ergostat upper-extremity ergometer. A 12-watt incremental protocol using 1-min stages was employed until the subject reached maximum effort, as determined by the following criteria: an R ($\dot{V}CO_2/\dot{V}O_2$) >1.10, HR ≥85% maximum predicted heart rate (MPHR = 220 − age), subjective exhaustion, or other limiting symptoms.

Determination of body composition was performed in the Body Composition Unit, Columbia/St. Luke's-Roosevelt Hospital Center, New York, NY. The percentage of body weight composed of lean tissue (%L) was determined by measurement of potassium (^{40}K) in a 4-Pi counting chamber (10). The $^{40}K/^{39}K$ ratio is constant and known, providing the total body potassium (TBK) with an accuracy of ±4%. The percentage of body weight

TABLE 1
Plasma Insulin and Glucose Levels
(Mean ± SEM)

	NL	IGT	DM
FPG (mg/dL)	91 ± 2	96 ± 3	97 ± 5
FPI (μU/mL)	10 ± 1	11 ± 2	9 ± 1
PPI (μU/mL)	96 ± 12	191 ± 38*	148 ± 43

NL, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus; FPG, fasting plasma glucose; FPI, fasting plasma insulin; PPI, peak plasma insulin.
* $p < 0.005$ between NL and IGT groups.

TABLE 2
Lipid Concentrations
(Mean ± SEM, mg/dL)

Serum lipids	Paraplegia	Control
HDL cholesterol	36 ± 1**	47 ± 1
Total cholesterol	193 ± 6*	212 ± 4
LDL cholesterol	126 ± 5	137 ± 4
Triglycerides	156 ± 16	130 ± 7

*$p < 0.01$, **$p < 0.0001$.

composed of fat (%F) was determined by dual photon x-ray absorptiometry (DPX) (11).

Study IV: Thallium-201 Myocardial Imaging after Upper-Body Ergometry Exercise. Single proton emission computerized tomography (SPECT) imaging using an Elscint gamma camera and instrumentation was performed on a subset of 19 subjects with paraplegia without known CAD, using a modified upper-body ergometry exercise stress test (12). Briefly, an upper-body exercise test protocol was developed to provide a progressive exertional level of exercise sufficient to elicit a heart rate ≥85% of MPHR. A Fleisch Universal Ergostat ergometer was used. A multistage protocol was designed with each stage of 3-min duration, and incremental increases of 12 watts per stage, with an initial workload of 36 watts. Exercise continued until test termination criteria were met. At the end of each stage, the subject stopped pedalling and blood pressure and 12-lead ECG tracing were obtained. This process continued until symptoms limited exercise performance or MPHR criteria were met. One minute before termination of exercise, thallium 201 was injected and the subject was instructed to continue until the end of the stage. Imaging of the heart began within 10 min of peak exercise. Resting images were performed 3 to 4 hours after exercise.

Statistical Analyses. All calculations and statistical analyses were performed using the STATVIEW software program. Data were expressed as the mean value ± SEM. ANOVA was used to determine significance of differences in basal plasma glucose and insulin concentrations, age according to group, and peak plasma insulin concentrations. Student's *t*-test was used to determine the significance of differences between groups for lipid determinations. Linear regression analyses and multiple regression analysis were performed to determine the relationships between insulin sensitivity and its determinants.

Results

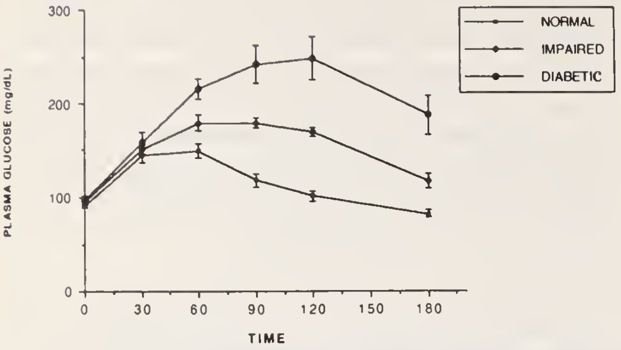
Study I: OGTT. The mean fasting plasma glucose concentration is higher in those with diabetes mellitus (97 mg/dL) than in those with normal glucose tolerance (91 mg/dL) (Table 1). However, no statistically significant difference is found among any of the groups. Mean fasting plasma insulin does not show any trend as glucose tolerance deteriorates (Table 1).

Of the 45 subjects with paraplegia evaluated by OGTT, 13 had impaired glucose tolerance and 9 had diabetes mellitus (Fig. 1). Subjects with normal glucose tolerance are significantly ($p < 0.05$) younger (43 ± 2 yrs) than those with impaired glucose tolerance (54 ± 3 yrs) or diabetes mellitus (58 ± 3 yrs). The peak plasma insulin level is higher in the groups with impaired glucose tolerance and diabetes mellitus than in the normal group, the difference being significant only in the comparison of subjects with impaired and normal glucose tolerance (Table 1).

The percentage of each group with a family history of diabetes mellitus increases as glucose tolerance worsens, 17% of those with normal tolerance, 25% of those with impaired glucose tolerance, and 33% of those with diabetes mellitus having a family history. The majority of subjects with paraplegia and impaired glucose tolerance or diabetes mellitus do not have a family history of a disorder of carbohydrate metabolism.

Study II: Serum Lipids. In serum lipid determinations in the 57 able-bodied controls and the 45 veterans with paraplegia (Table 2), mean LDL cholesterol is not significantly different, but mean total cholesterol and HDL cholesterol are significantly lower in those with paraplegia.

Almost half (48%) of the subjects with paraplegia have HDL cholesterol less than 35 mg/dL, and in 20% of those subjects the level is less than 30 mg/dL. Although only 13% of those with paraplegia have LDL cholesterol greater than 160 mg/dL, a total of 41% have levels greater than 130 mg/dL. Thirty-six percent of the subjects with paraplegia with HDL cholesterol less than 35 mg/dL have LDL cholesterol greater than 130 mg/dL.



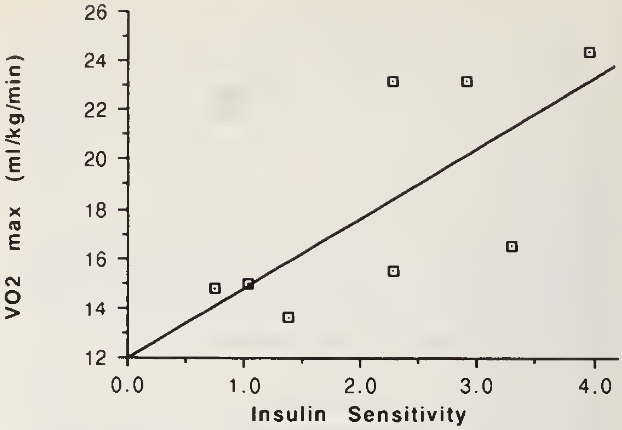


Fig. 1. **Left** Oral glucose tolerance test results by diagnosis plotted as linear graph. Results are expressed as mean ± SEM.
Fig. 2. **Right** Correlation between insulin sensitivity and cardiovascular fitness. Insulin sensitivity determined by minimal-model method (7). Cardiovascular fitness was determined from a metabolic upper-body ergometry stress test with measurement of $\dot{V}O_2$ max (8, 9).

Study III: Determinations of Insulin Sensitivity, Body Composition, and Cardiopulmonary Fitness. The correlation of insulin sensitivity (S_i) with body composition and maximal aerobic power ($\dot{V}O_2$ max) was tested in 8 subjects with paraplegia and a mean age of 44 ± 4 years, body weight of 83.0 ± 5.4 kg, BMI of 26.5 ± 1.2, and duration of injury 10 ± 2 years. In this subgroup, 5 have normal glucose tolerance, 1 has impaired glucose tolerance, and 2 have diabetes mellitus. The mean S_i is 2.2 ± 0.4, range 0.8–4.0, consistent with a state of insulin resistance (7).

The linear correlation between S_i and %L is positive. The equation for the line is: $y = 0.098x - 2.134, p = 0.07$. The mean total body lean is 44 ± 3 kg (about one-third below predicted), range 36–60 kg. The linear correlation between S_i and %F is negative. The equation for the line is: $y = -0.063x + 4.217, p = 0.25$. The mean total body fat is 34 ± 3 kg, range 23–48 kg. The mean %F is 31 ± 3, range 21%–46%.

The linear correlation between S_i and cardiovascular fitness ($\dot{V}O_2$ max) is positive (Fig. 2). The equation for the line is: $y = 0.178x - 1.011$, $r = 0.71$, $p < 0.05$. Mean $\dot{V}O_2$ max is 19 ± 2 mL/kg · min (range 14–24).

The combined effect of %L, %F, and $\dot{V}O_2$ max on S_i was calculated by using multiple regression analysis. The multivariate correlation coefficient is 0.82. The regression equation obtained for the predicted S_i is: $S_i = (0.021) \cdot (\%L) - (0.042) \cdot (\%F) + (0.155) \cdot (\dot{V}O_2 \text{ max}) - 0.226$.

Study IV: Thallium-201 Myocardial Imaging after Upper-Body Ergometry Exercise. Radionuclide myocardial imaging after upper-body ergometry was performed on 19 subjects with paraplegia, mean age 54 ± 3 years, BMI 26 ± 3 kg/n^2, duration of injury 17 ± 3 years. Five (of 19) had a positive stress test using electrocardiographic (ECG) results alone. Twelve (of 19), about 60%, had a positive study from radionuclide perfusion imaging; thus, an ECG stress test alone would have missed 55% of subjects with myocardial ischemia:

- 4/19 had single-wall ischemia
- 7/19 had multiple-wall ischemia
- 5/19 had silent myocardial infarctions, 3 with single wall and 2 with multiple wall infarcts, 1 of whom had no evidence of ischemia

Discussion

There are approximately one-quarter of a million individuals with SCI in the United States, with an incidence of approximately 10,000 new cases per year (13). As in nondisabled individuals, CAD is a leading cause of morbidity and mortality. Cardiovascular disease deaths occur at relatively younger ages in persons with SCI than in the able-bodied (1). This report demonstrates a high prevalence of metabolic factors which have been recognized to be risk factors of CAD, and, furthermore, it demonstrates a high prevalence of latent CAD.

Impaired glucose tolerance and diabetes mellitus are associated with an increased risk of cardiovascular disease (14). Patients with SCI have been reported to have a greatly increased prevalence of abnormalities in carbohydrate metabolism (15). In an ambulatory population, prolonged inactivity has been shown to impair glucose tolerance, with associated hyperinsulinemia (16), and even mild degrees of physical activity improve glucose tolerance (17). Both sedentary life style and increased adiposity have individually and in combination been implicated in causing insulin resistance and in decreasing the ability to handle a carbohydrate load (18, 19). Despite the fact that patients with SCI may have both these predisposing factors, the relative prevalence of and causes for impaired carbohydrate handling in these patients has not been fully investigated. A well-recognized risk factor of CAD in able-bodied individuals, lipid disorders have been little studied in those with SCI. Low plasma HDL cholesterol levels in relatively young individuals with SCI have been recognized in a few recent reports (20–22). However, middle-aged individuals with SCI have not been investigated for lipoprotein profiles, nor for the association between carbohydrate and lipid metabolism.

Duckworth et al. (15) demonstrated that 23 of 41 patients with SCI were diabetic by 100-g OGTT in spite of normal fasting glucose levels. Twelve of the 23 diabetic patients were shown to be hyperinsulinemic during OGTT, and 7 of these had a blunted response to exogenous insulin. Thus, diabetes was noted in 56% of subjects with SCI, and about half of these were insulin resistant and hyperinsulinemic. No attempt was made by these investigators (15) to differentiate the response to testing between those with paraplegia or quadriplegia. In this report, we demonstrated that in 45 healthy outpatient subjects with paraplegia, almost half had an abnormality in carbohydrate tolerance and 20% were classified as having diabetes mellitus.

Spinal lesions of longer duration were generally associated with insulin resistance (15). However, the etiology of insulin resistance in patients with SCI has not been fully explained. Obesity is known to be associated with insulin resistance. In the Duckworth et al. study (15), the insulin-resistant patients with SCI were more obese than those who were insulin-sensitive (as determined from actuarial charts of body weight and by BMI), but 6 of 12 insulin-resistant patients were not apparently obese by these established criteria. However, BMI measurements have not been validated as surrogate measurements for obesity in patients with SCI, raising a question concerning their applicability. Furthermore, the degree of hyperinsulinemia in these 6 subjects far exceeded that predicted solely on the basis of body weight and estimated percentage of body fat (15). In this report, the group comprised of those with paraplegia and impaired glucose tolerance had significantly higher plasma insulin levels than those with normal glucose tolerance; the diabetic group had higher plasma insulin levels than the normal group, but the difference did not reach statistical significance, possibly due to the relatively small number of subjects in the groups. These findings for plasma insulin in persons with SCI are quite similar to those for able-bodied veterans with varying degrees of glucose intolerance as reviewed by Yalow et al (23).

Jarvinen (24) studied the effects of body composition and $\dot{V}O_2$ max on the rate of glucose metabolism in males of normal weight who were either weight lifters, long-distance runners, or untrained controls. The strongest determinant of the predicted rate of glucose metabolism was body composition, and, more specifically, body fat. In our study group, the level of activity or cardiopulmonary fitness, not body adiposity, was found to be the strongest determinant of insulin sensitivity in untrained subjects with paraplegia.

Recent reports have revealed lower HDL cholesterol levels in patients with SCI (20–22), which is also a feature of the obese diabetic (25). We have reported herein that HDL cholesterol is markedly depressed in our healthy outpatient subjects with paraplegia. If a lipoprotein profile were performed solely on the basis of an elevated total cholesterol, 89% of those with low HDL cholesterol, an independent risk factor of CAD, would have been missed. Furthermore, about one-third of those with paraplegia with low HDL cholesterol had LDL cholesterol greater than 130 mg/dL, a level relatively elevated if 2 or more CAD risk factors are present that may require specific dietary or pharmacologic intervention to lower. The Framingham Study (26, 27) established an inverse relationship between HDL cholesterol and CAD. Brenes et al. (20) reported that in 56 relatively young and inactive male subjects with SCI, mean HDL cholesterol was 35 mg/dL, whereas in 22 highly trained wheelchair athletes the mean HDL cholesterol was 43 mg/dL. Dearwater et al. (21) noted that a similar group of young, inactive subjects with SCI had mean HDL cholesterol levels of 34 mg/dL, whereas a group of wheelchair athletes had significantly higher values. These investigators (21) noted that serum glucose and basal insulin levels were similar in the groups with SCI, regardless of level of activity.

Finally, since there is a clustering of metabolic factors which predispose to CAD, it is not unexpected that we observed a high prevalence of latent CAD in subjects with paraplegia. Many subjects with paraplegia, despite performing the chores of daily living, may have subclinical myocardial ischemia, and thus undetected CAD. Radionuclide myocardial imaging may be especially useful in patients with SCI who are to undergo noncardiac operative procedures, which are greatly increased in this population, and in whom the morbidity and mortality from coronary-related events is increased. In the absence of radionuclide imaging, cardiac evaluation to diagnose CAD would be possible only by the use of cardiac catheterization with coronary angiography, invasive techniques that would generally not be employed in the absence of symptoms. The full implications of radionuclide-diagnosed CAD in asymptomatic subjects with paraplegia have yet to be addressed.

References

1. Whiteneck G. Learning from empirical investigations. In: Menter R, Whiteneck G, eds. Perspectives on aging with spinal cord injury. New York: Demos Publ., 1992.
2. Kennedy EJ, Stover SL, eds. Spinal cord injury, the facts and figures. Birmingham, Ala: US National Spinal Cord Injury Statistical Center, University of Alabama, 1986.
3. Samsa P, Patrick C, Feussner JR. A national study of mortality among spinal cord injured veterans. IIR No. 87-036.
4. Berson SA, Yalow RS. General radioimmunoassay. In: Investigative and diagnostic endocrinology. Amsterdam: North-Holland Publishing Co., 1973:84–120.
5. Yalow RS, Berson SA. Radioimmunoassay of insulin. In: Methods in investigative and diagnostic endocrinology. Amsterdam: North-Holland Publishing Co., 1973:864–870.
6. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28:1039–1040.
7. Bergman RN, Praguer R, Volund A, Olefsky JM. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. J Clin Invest 1987; 79:790–800.
8. Astrand P, Rodahl K. Evaluation of physical work capacity on the basis of tests. In: Textbook of work physiology, 2nd ed. New York: McGraw-Hill, 1977:331–338.
9. Wasserman K, Hansen J, Sue D, Whipp B. Physiology of exercise. In: Principles of exercise testing and interpretation. Philadelphia: Lea & Febiger, 1987:3–26.
10. Pierson RN, Jr, Wang J, Thornton J, Van Itallie TTB. Body composition by 4-P_i counting: an anthropometric correction. Am J Physiol 1982; 246:F234–239.
11. Russell-Aulet M, Wang J, Thornton J, Pierson RN, Jr. Comparison of dual photon absorptiometry systems for total body bone and soft tissue measurements: dual energy x-ray versus gadolinium-153. J Bone Miner Res 1991; 6:411–415.
12. Spungen AM, Bauman WA. A protocol for performing arm-crank ergometry thallium cardiac stress testing in paraplegics. Clin Res 1991; 39(2):406A.
13. Young JS, Burns PE, Bowen AM, McCutchen R. Demography. In: Spinal cord injury statistics: experience of the regional spinal cord injury systems. Phoenix, Ariz: Good Samaritan Medical Center, 1982:13–25.
14. Welborn TA, Wearne K. Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. Diabetes Care 1979; 2:154–160.
15. Duckworth WC, Solomon SS, Jallepalli P, Heckemeyer C, Finnern J, Powers A. Glucose intolerance due to insulin resistance in patients with spinal cord injuries. Diabetes 1980; 29:906–910.
16. Lipman RL, Schnure JJ, Bradley EM, Lecocq FR. Impairment of peripheral glucose utilization in normal subjects by prolonged bed rest. J Lab Clin Med 1970; 76:221–230.
17. Lipman RL, Raskin P, Love T, Triebwasser J, Lecocq F, Schnure JJ. Glucose intolerance during decreased physical activity in man. Diabetes 1972; 21:101–107.
18. Krotkiewski M, Mandroukas M, Sjostrom LM, Sullivan H, Witterquist H, Bjorntorp P. Effects of long-term physical training on body fat, metabolism and blood pressure in obesity. Metabolism 1979; 28:650–658.
19. Yalow RS, Glick SM, Roth J, Berson SA. Plasma insulin and growth hormone levels in obesity and diabetes. Ann NY Acad Sci 1965; 131:357–373.
20. Brenes G, Dearwater S, Shapera R, LaPorte RE, Collins E. High density lipoprotein cholesterol concentrations in physically active and sedentary spinal cord injured patients. Arch Phys Med Rehabil 1986; 67:445–450.
21. Dearwater SR, LaPorte RE, Robertson RJ, Brenes G, Adams LL, Becker D. Activity in the spinal cord-injured patient: an epidemiologic analysis of metabolic parameters. Med Sci Sports Exerc 1986; 18:541–544.
22. Hooker SP, Wells CL. Effects of low- and moderate-intensity training in spinal cord-injured persons. Med Sci Sports Exerc 1989; 21:18–22.
23. Yalow RS, Rose HG, Bauman WA. Hyperinsulinemia. Am J Med 1988; 85:22–30.
24. Jarvinen HY, Koivisto VA. Effects of body composition on insulin sensitivity. Diabetes 1983; 32:965–969.
25. Abrams JJ, Ginsberg H, Grundy SM. Metabolism of cholesterol and plasma triglycerides in nonketotic diabetes mellitus. Diabetes 1982; 31:903–910.
26. Castelli WP. Epidemiology of coronary heart disease: The Framingham study. Am J Med 1984; 76(2A):4–12.
27. Castelli WP, Leaf A. Identification and assessment of cardiac risk—an overview. Cardiol Clin 1985; 3(2):171–178.

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society

Organ Response and Cell Development

The Response of the Myocardium to Overload Stress

Sidney S. Schreiber, M.D., M.Sc.

Proteins are an essential part of all living cells. These include enzymes for energy requirements, for the structural integrity of cells and their membranes, for nucleoproteins and the protein synthetic machinery itself, for the integrity of the nuclei and chromatin, and in certain cells, such as cardiac muscle, the contractile mechanism which is the fundamental part of the cardiac pump. Although in the living organism, cells such as myocardial myocytes may not reproduce, the proteins are constantly being replaced. Therefore, an essential process in the life of any cell is the synthesis of proteins.

One of the major findings in the study of such synthesis has been the relatively rapid turnover (synthesis and degradation) of proteins even in the complex myocardial cell. This rapid turnover of proteins has permitted the study of the response of the cardiac pump to various stresses.

One of the stresses we have examined for several years has been the induction of increased resistance to cardiac outflow (increased afterload), commonly occurring in valvular resistance or arterial hypertension. The result, which is well known, is enlargement of the heart with increased muscle mass or hypertrophy of the stressed ventricle. The mechanism of induction of the hypertrophy has been the main subject of study in our laboratory, and it has been found that the primary response to increased stress is augmented protein synthesis (1), which leads to hypertrophy.

Problems in Assay of Protein Synthesis

A change in the rate of protein synthesis may be determined from the incorporation of a labeled amino acid into the protein(s) of interest (2, 3). By definition, the concentration of radioactive labeled amino acid (for example, in microcuries), divided by the concentration of labeled and unlabeled amino acid (for example, in micromoles), is known as "specific activity." If a specific protein is being measured, the quantity of activity incorporated into a unit weight of protein in any specific time period, divided by the specific activity of the labeled amino acid, equals the quantity of amino acid incorporated into the unit weight of the protein in the period of study. If a specific protein is being studied, the quantity of amino acid incorporated, divided by the amount of amino acid in the protein molecule, will give the amount of protein synthesized.

Although the general idea appears simple, the measurement of protein synthesis has often been beset with difficulties both in the steady state and in changing stresses (3). The mechanism of protein synthesis requires that an individual amino acid is esterified to a specific transfer RNA (tRNA) and inserted into a growing polypeptide chain. The tRNA pool of labeled amino acid is called the "precursor pool."

For the assay of protein synthesis to be valid, the activity or the concentration of radioactive labeled amino acid in the tRNA pool must be constant, or nearly so. In early studies with labeled amino acids, the specific activity of the amino acid used in calculations of protein synthesis was

---

From the Department of Clinical Medicine, New York University School of Medicine, and the Department of Nuclear Medicine, Department of Veterans Affairs Medical Center, New York, NY. Address reprint requests to the author, Professor of Clinical Medicine, New York University School of Medicine, and Attending Internist, Department of Nuclear Medicine, Veterans Affairs Medical Center, 423 E. 23rd Street, New York, NY 10010.

taken to be that in the infusion both in vivo and in vitro (4, 5). However, defects in the system became apparent, since the mode of administration of the labeled amino acid will influence the specific activity of the amino acid in the precursor (tRNA) pool. For example, after a single injection of the labeled amino acid in vivo (pulse label), there are continuously changing levels of the radioactive label in the plasma, extracellular and intracellular space, and, finally, in the tRNA pool. To estimate synthesis of a protein, complex mathematics and repeated sampling of the spaces and cardiac tissue (which is not feasible), is needed. Continuous perfusion with constant concentration of the labeled amino acid in studies in vivo and in vitro has permitted more accurate estimates of protein synthesis and is applicable in experiments with induced stress to the heart.

The second problem concerned which compartment was to be used as an index of the precursor pool, since the specific activity of the labeled amino acid in the perfusate and extracellular spaces, intracellular space, and the tRNA pool were not necessarily the same. In our early studies with the isolated guinea pig heart and labeled lysine, the calculations of synthesis were based on the specific activity of the labeled amino acid in the circulating perfusate (5). Subsequent data showed that the specific activity in the intracellular cardiac pools was lower than in the perfusate, and these values were used in later studies. To complete the circle, more recent experiments demonstrated that the tRNA pool for lysine had a specific activity which more closely approximated that in the extracellular space or perfusate than that in the intracellular spaces (6).

Response to Hypertensive Stress

Since we were interested in the early response of the myocardium to stress, we began our studies with hearts perfused in vitro (5). There were two main advantages to in vitro studies. First, the pressure or outflow resistance as well as cardiac flow could be controlled. Second, the assay of synthesis was simplified by using a constant concentration of radionuclide in the perfusate. Induction of outflow resistance resulted in increased incorporation of labeled lysine into total cardiac protein. A time-course study showed that significant increases in synthesis of total left ventricular protein was evident as early as 2–3 h after initiation of stress.

Once we had established that protein synthesis rapidly responded to hypertensive stress applied to the left ventricle, we began to trace the factors initiating this response with studies using subcellular fractions. Since distinct increases in protein incorporation of labeled lysine was seen as early as 2–3 h after initiation of the stress, the synthetic factory must have been in operation before then.

TABLE 1

Incorporation of 14C-Lysine into Cardiac Microsomes

C-C†	C-E†	E-C†	E-E†
100	117	263*	281*

† Microsome-cell sap mixtures; first letter, microsome; second letter, cell sap. C, control, E, overloaded.

* Significantly different from C-C and C-E, $P < .001$.

See ref. 7.

Studies with Subcellular Systems. After left ventricles of isolated guinea pig hearts were overloaded by increasing aortic pressure for one hour, microsomes were isolated and incubated in appropriate mixtures and labeled amino acids (7). After incubation, proteins were isolated, and incorporation of radioactive amino acids into the proteins assayed. Cross-incubation studies revealed that microsomes from overloaded hearts showed increased synthesis of proteins, whether they were incubated in their own cytoplasm (cell sap) or in cytoplasm from control hearts (Table 1). Subsequent studies demonstrated that incubation of microsomes from such stressed hearts showed increased synthesis of proteins even when incubated in cytoplasm from other cells such as hepatocytes.

The data so far indicated that acute overload in the young guinea pig heart stimulated an increase in protein synthesis as early as one hour after onset of this particular stress. However, the specific parts of the microsome involved in this process was not clear. Our first clue was obtained from the study of the effect of actinomycin-D, an inhibitor of RNA synthesis. Hearts taken from animals pretreated with actinomycin-D and perfused with the same agent were able to perform normally, and protein synthesis was the same as in control untreated hearts, indicating that actinomycin did not damage the protein synthetic capacity per se. Under the stress of increased afterload, the actinomycin-treated hearts functioned without difficulty, but synthesis of proteins in these stressed ventricles did not increase with hemodynamic overload and was no greater than in control hearts. Thus, with actinomycin there was significant inhibition of incorporation of labeled amino acids into new protein synthesis in microsomes from the overloaded hearts (Table 2) (8).

The studies with actinomycin strongly sug-

TABLE 2
Effect of Actinomycin-D on Incorporation of Labeled Leucine into New Protein by Microsomes from Control and Overloaded Ventricles

Control	Control plus A	Overloaded	Overloaded plus A
100	99	219*	115**

A, actinomycin.
Control microsome synthesis was set at 100.
* $P < .001$.
** Not significant.
See ref. 7, 16.

gested that the initial effect leading to increased protein synthesis in overload involved synthesis of RNA. However, it was unclear whether the initial response was a stimulation of formation of "new" mRNA (trascription), release of preformed mRNA, increased synthesis and population of ribosomes, or increased translation of available ribosomes on preexisting mRNA (9).

In a relatively simplistic experiment, an artificial messenger, polyuridylic acid, was added to microsomes from control and overloaded ventricles treated and untreated with actinomycin (8). In this type of study, incorporation of labeled phenylalanine into the protein fraction is used as an index of protein synthesis (peptide formation). Addition of polyuridylic acid to microsome mixtures from control ventricles resulted in increased incorporation of the labeled amino acid into protein, indicating available functioning ribosomes unbound to mRNA. Microsome mixtures from overloaded ventricles showed increased incorporation of phenylalanine into protein as expected, but the addition of polyuridylic acid did not show any further increments.

This suggested that the microsome mixture from overloaded ventricles contained ribosomes already bound to mRNA, newly formed or released, rather than an increase in ribosome population. This was further supported by the addition of polyuridylic acid to microsome mixtures from hemodynamically overloaded hearts treated with actinomycin. In such mixtures, polyuridylic acid increased incorporation of phenylalanine as in controls and indicated an abundance of free ribosomes unlike the microsomes taken from untreated overloaded ventricles.

The findings supported the general thesis that acutely overloaded ventricles stressed with increased pressure responded with an early increase in RNA (8, 9), probably initially with messenger variety (8).

Further studies of early synthesis of ventricular mRNA following overload were carried out with the perfusion of stressed ventricles with a labeled precursor of RNA synthesis, ^{3}H-uridine (10). After 1 hour of perfusion, polysome profiles of overloaded ventricles showed increased labeling of heavier aggregates than in control ventricular microsomes. Ribosome labeling, per se, was the same in both control and overloaded. Labeling of the heavier aggregates in overloaded microsomes was abolished by actinomycin (8). The evidence further suggested an early increase in mRNA rather than an increase in number of ribosomes or in ribosomal RNA following hypertensive overload.

Studies with Isolated Nuclei. The changes mentioned above with microsomes taken from overloaded ventricles were noted as early as one hour following pressure stress in the isolated perfused heart. It was evident that if synthesis of new mRNA did actually occur, this response should have been seen even earlier after onset of the overload. For this reason, we began our studies with nuclei isolated from ventricles in hearts perfused in vitro at control or overload pressures. At the completion of perfusion, nuclei were isolated, purified, and then suspended in Mg^{++} or Mn^{++} media containing ^{32}P-labeled UTP. By definition, Mn^{++} media stimulated nuclear polymerase activity synthesizing mRNA, whereas Mg^{++} media stimulated synthesis mainly of ribosomal RNA (11, 12). The data obtained from ventricles of hearts perfused for 20–120 min showed an initial significant increase in Mn^{++}-stimulated nuclear RNA polymerase II activity (synthesizing mRNA), and after one hour a gradual rise in the Mg^{++} nuclear RNA polymerase activity (synthesizing ribosomal RNA) (13). A delayed rise in synthesis of rRNA was also described in other models (14).

The polymerase studies with isolated nuclei were taken one step further. Nuclei were obtained from normal nonperfused hearts by centrifugation through high-density sucrose gradients. Microscopic examination has indicated that 90% of the nuclei are typical of myocyte nuclei. The nuclei were then suspended in media containing ^{3}H-UTP and subjected to increased hydrostatic pressure for 20–30 min (15). After 20 min of pressure, Mn^{++}-stimulated RNA polymerase II activity was increased, whereas Mg^{++} activity was actually slightly depressed or unchanged. The response of RNA polymerase II activity to pressure was completely inhibited by alpha-amanitin in concentrations known to inhibit synthesis of mRNA by RNA polymerase II activity.

The data were therefore highly suggestive that direct pressure to nuclei could elicit the response of increased mRNA synthesis (15, 16). Thus, it was tempting to suggest that pressure itself on the myocyte nuclei stimulated nuclear RNA polymerase II activity, which in turn formed new mRNA, but the pressure transducer was and still is unknown. Recently, it has been suggested that deformity or stretching of the nuclear membrane may play a role (17).

The sequence of events following acute overload appeared to be as follows: nuclear stimulation to form mRNA, release of "new" mRNA to the available ribosomes, subsequent increases in rRNA, and the formation of protein. A clue to which proteins were initially formed in overload was suggested by early studies on synthesis of contractile proteins. It was found that a preferential synthesis of myosin heavy chains took place after two to three hours (18). This finding fits in with subsequent work in several laboratories which showed that the heavy chains in rat hearts have two isomers, an alpha and a beta form, and that it is the beta myosin HC which apparently increased in pressure loading (19, 20). The data appear to fit the sequence of early events described above. It is clear, however, that to produce a functioning myofibril in the presence of persistent overload, eventually an increase occurs in synthesis of all the myocyte proteins.

Studies with Heterotopic Transplanted Hearts. Recently, our studies of the responses of the myocardium to the stress of work have involved the heterotopic transplanted rat heart. These studies were done with the collaboration of Irwin Klein and Chull Hong (21). It is known that the heart transplanted to the abdominal aorta may receive coronary flow with the same pressure as the host heart, but the left ventricle performs little work of ejection. In essence the transplant is an in vivo Langendorff perfused heart. It has been shown that after transplantation, although the transplant survives, continues to beat regularly, and shows little if any damage, there is atrophy until a new steady state has been reached, by 2–3 weeks. In contrast, the host heart continues to grow during this period. Data obtained with animals (hosts with heterotopic transplanted hearts) at four periods after transplantation (n = 6 for each period) (Table 3) included a fall in donor heart weight which was due to a decline of protein synthesis during this period, although the transplanted hearts survived and beat without difficulty. There was no evidence of myocyte damage when the transplants were studied histologically.

TABLE 3

Weights and Synthesis in Transplanted Nonworking Hearts

	Days			
	0 (n = 6)	3 (n = 6)	7 (n = 6)	14 (n = 6)
Weight (g)				
Host	425 ± 8	467 ± 9	570 ± 18	632 ± 16
Heterotopic		379 ± 5	322 ± 12	295 ± 14
Synthesis*				
Host	50 ± 4	42 ± 3	64 ± 3	69 ± 6
Heterotopic		21 ± 4	25 ± 1	22 ± 3

* Protein synthesis expressed as incorporation of ^{14}C-labeled lysine into protein (umol lysine/ventricle · h).
Difference between host and heterotopic hearts significant at 3, 7, & 14 days ($P < .05$ for synthesis; $P < .001$ for weights).
See ref. 21.

To demonstrate that the atrophy of the transplanted hearts was not due to ischemia incurred during the transplantation procedure or to deprivation of neurogenic control, further studies were carried out with this preparation utilizing intraventricular distended balloons (22). At the time of transplantation, a specially designed latex balloon was inserted into the left ventricles of the transplant and filled with saline to a volume of 0.08–0.1 mL, which visibly distended the left ventricle. The balloon was closed and then secured in the ventricle and the animal closed. Coronary flow to the hearts was maintained as in transplants without balloons. Controls were run with balloons inserted into the ventricle but without any distention. Thus, from the time of transplantation the left ventricles with distended balloons were subject to augmented work of isometric type.

The data obtained indicated that with the introduction of a load to the left ventricle of the heterotopic transplants, there was no left ventricular atrophy (Table 4).

Summary and Conclusions

Our studies have demonstrated that the sequence of events following acute hemodynamic

TABLE 4

Intraventricular Balloons and Left Ventricle (LV) of Transplants

14 days	LV weight (g)	LV Synthesis*
Host heart	0.53 ± 0.01	49 ± 6
Transplanted		
Unloaded	0.23 ± 0.01	22 ± 3
Sham loaded	0.23 ± 0.02	21 ± 3
Balloon loaded	0.40 ± 0.01	40 ± 4

* Mean nmols lysine/LV · hr ± SEM
Weights of ballooned left ventricles and synthesis were significantly greater than unloaded heterotopic and sham-loaded heterotopic transplants.
See ref. 22.

overload appears to originate in the nucleus with the increased formation of mRNA, followed by synthesis of rRNA and by increased activity in subcellular systems, finally resulting in increased protein synthesis and ventricular mass. The transducer transmitting physical force to nuclear and DNA response is still not known but may involve stretching of the nuclear membrane (17) which invokes cyclic AMP stimulation of protein synthesis (23, 24).

The final product of genetic expression is protein synthesis. It has been presumed that a growing organ, such as the heart, assumes size and shape as programmed, but it is known, at least with cardiac and skeletal muscle, that workloads augment growth within genetic limits. Our data with the nonworking and ballooned left ventricles of transplanted hearts indicate that full genetic expression in the growing heart depends on external influences such as work load. If we may extrapolate to the future, the findings also suggest that a danger to cardiac development may be present in children growing in a gravity-free environment such as a space station without the introduction of artificial gravitation and a cardiac workload.

Acknowledgments

Supported in part by the National Heart Institute HL 09562, the Veterans Administration, and the Berger Foundation.

References

1. Schreiber SS, Evans CD, Oratz M, Rothschild MA. Protein synthesis and degradation in cardiac stress. Circ Res 1981; 48:601–611.
2. Garlick PJ. An analysis of errors in estimation of the rate of protein synthesis by constant infusion of a labeled amino acid. Biochem J 1978; 176:402–405.
3. Schreiber SS, Evans CD, Oratz M, Rothschild MA. Problems in evaluating cardiac protein synthesis. J Mol Cell Cardiol 1982; 14:307–312.
4. Morgan HE, Chua BHL, Fuller EO, Siehl D. Regulation of protein synthesis and degradation during in vitro cardiac work. Am J Physiol 1980; 239:E431–442.
5. Schreiber SS, Oratz M, Rothschild MA. Protein synthesis in the overloaded mammalian heart. Am J Physiol 1966; 211:314–318.
6. Evans CD, Schreiber SS, Oratz M, Rothschild MA. Relative synthesis of cardiac contractile proteins. Biochem J 1981; 194:673–678.
7. Schreiber SS, Oratz M, Rothschild MA. Effect of acute overload on protein synthesis in cardiac muscle microsomes. Am J Physiol 1967; 213:1552–1555.
8. Schreiber SS, Oratz M, Evans CD, Silver E, Rothschild MA. Effect of acute overload on cardiac muscle mRNA. Am J Physiol 1968; 215:1250–1259.
9. Nair KG, Cutiletta AF, Zak R, Koide T, Rabinowitz M. Biochemical correlates of cardiac hypertrophy. Circ Res 1968; 23:451–462.
10. Schreiber SS, Oratz M, Rothschild MA. Nuclear RNA polymerase activity in acute hemodynamic overload in the perfused heart. Am J Physiol 1969; 217:1305–1309.
11. Lindell TJ, Weinberg F, Morris PW, Roeder RG, Rutter WJ. Specific inhibition of nuclear RNA polymerase II by alpha amanitin. Science 1970; 170:447–449.
12. Versteegh LR, Hearn TF, Warner CM. Variations in amounts of RNA polymerase I, II, & III during preimplantation development in the mouse. Dev Biol 1975; 46:430–435.
13. Schreiber SS, Oratz M, Rothschild MA. Nuclear RNA polymerase activity in acute hemodynamic overload in the perfused heart. Am J Physiol 1969; 217:1305–1309.
14. Nair KG, Rabinowitz M, Chen Tu M. Characterization of ribonucleic acid synthesized in an isolated nuclear system from rat heart muscle. Biochemistry 1967; 6:1899–1902.
15. Schreiber SS, Oratz M, Rothschild MA, Reff F. Effect of hydrostatic pressure on isolated cardiac nuclei: stimulation of RNA polymerase II activity. Cardiovasc Res 1978; 12:265–268.
16. Schreiber SS, Evans CD, Oratz M, Rothschild MA. Cardiac protein synthesis in stress. Raven Press, New York 1983:452–463.
17. Mann DL, Kent RL, Cooper Y. Load regulation of the properties of feline cardiocytes: Growth induction by cellular deformation. Circ Res 1989; 1079–1090.
18. Evans CD, Schreiber SS, Oratz M, Rothschild MA. Synthesis of myosin heavy and light chains in the afterloaded guinea pig right ventricle. Cardiovasc Res 1978; 12:731–743.
19. Hoh JFY, Yeoph GPS, Thomas MAW, Higginbottam L. Structural differences in the heavy chain of rat ventricular myosin isozymes. FEBS Lett 1979; 97:330–334.
20. Nagai R, Pritzel N, Low RB, Stirwalt WS, Zak R, Alpert N, Litten R. Myosin isozyme synthesis and mRNA levels in pressure overloaded rabbit hearts. Circ Res 1987; 60:692–699.
21. Klein I, Hong C, Schreiber SS. Cardiac atrophy in the heterotopically transplanted rat heart: in vitro protein synthesis. J Mol Cell Cardiol 1990; 461–468.
22. Klein I, Hong C, Schreiber SS. Isovolumic loading prevents atrophy of the heterotopically transplanted rat heart. Circ Res 1991; in press.
23. Schreiber SS, Klein I, Oratz M, Rothschild MA. Adenylcyclase activity and cyclic AMP in acute cardiac overload: a method for measuring cyclic AMP production based on ATP specific activity. J Mol Cell Cardiol 1971; 2:55–65.
24. Xenophontas XP, Watson PA, Chua B, Haneda T, Morgan HE. Increased cyclic AMP content accelerates protein synthesis in the rat heart. Circ Res 1989; 65:647–656.

Development of the Beta Cells

B. Bréant, C. Lavergne, A. Astesano, N. Ferrand, M. Asfari, C. Boissard, A. Anteunis, and G. Rosselin

Understanding of the regulation of the main β-cell functions has progressed significantly through studies on insulin synthesis and secretion initiated by the discovery of radioimmunoassay (1). Whereas most of the β-cell receptors involved in regulation of insulin release are now characterized (2), few works examine regulation of β-cell growth (3–7), and the origin and mechanism of duplication of β cells are largely unknown. But the processes responsible for cell growth and differentiation are also known to be regulated by environmental signals (8, 9). β-cell growth is probably governed by many substances, including growth factors as well as neuroendocrine and paracrine mediators interacting with specific cell surface receptors. Their effects are transduced from the membrane to the final site of action by multicomponent signalling pathways. Most β cells remain in a resting state, also called the G0 phase, and continue to exert their differentiated functions. Appropriate environmental signals can induce these cells to reenter the G1 phase of the cell cycle, from which they progress toward the S phase. Once their DNA content is doubled, they enter the G2 phase and thereafter undergo mitosis. Alternatively, dividing precursor cells may, through further differentiation, participate in the production of functional β cells. The development of different pools of cells through these two cellular processes may occur simultaneously.

In this paper, we initially focus on the regulation of β-cell growth, which may be controlled by external signals or autocrine processes, depending on the cell line. In the second part we study the cell types involved at the onset of the β-cell regeneration that follows streptozotocin-induced diabetes. We demonstrate in situ the existence of a new β-cell subpopulation likely to be involved in β-cell regeneration by further differentiation.

Regulation of β-Cell Growth

Normal β Cells and Growth. Several in vivo experiments (see review of Hellerström [7]) indicate the functional adaptation of β-cell growth to environmental factors. Replication of β cells is more rapid in fetus and neonates than in adults; however, persistent replication occurs in adults. Calculations from autoradiography following continuous administration of [3H] thymidine to adult mice indicated that islet cells are renewed at the rate of 0.14 to 0.2%/day (5). The replication of β cells increases with high food intake, a carbohydrate-rich diet, and glucose levels. Evidence for the role of glucose was obtained by raising glucose levels through either short-term glucose infusion or injection of anti-insulin antibodies (5, 10). β-cell proliferation is also higher in genetic or nutritional obesity than in corresponding controls. It can also be stimulated by growth hormone, cortisol (7), and bombesin (11). It was not found to vary in an experimental model of diabetes (12). Most of the in vitro studies on the regulation of β-cell growth performed with primary cultures of islets (3) substantiate that β-cell proliferation is variably stimulated by glucose (13), and also by growth hormone through IGF-1 induction (14). These studies gave invaluable results but had several limitations: β cells in islets are clustered in a structure organized nonrandomly, giving islets an architecture that is convenient for circumscribed paracrine effects and cell-to-cell functional interrelationship. Consequently, the induced progression of β cells in the cell division cycle cannot be directly attributed to the added effectors. Furthermore, growth-restricting factors are likely to be present in islets,

---

From the Institut National de La Santé et de La Recherche Médicale, Unité de Recherches sur Les Peptides Neurodigestifs et Le Diabète, Unite 55. Address reprint requests to G. Rosselin at Centre de Recherches Paris Saint-Antoine, 184, rue du Faubourg Saint-Antoine, Paris Cedex 12.

as shown by the high ratio of resting cells in mature islets.

β-Cell Lines. In the past, several pancreatic β-cell lines have been established. Some were obtained from transplantable hamster insulinoma (15), others were RIN cells derived from insulinoma following X-ray irradiation and transplantation in the rat (RINr cells) or mouse (RINm cells) (16); several clones of tumoral cells were obtained (4, 16, 17). The first glucose-responsive β-cell clones were selected from a hamster cell line obtained by transformation of normal islets (18) with simian virus 40 (SV-40). More recently, several transgenic mouse β-cell lineages were isolated; they derived from primary culture of islet tumors (19) that developed in transgenic mice expressing the SV-40 large T-antigen on the control of the rat insulin II promoter (20). The latter directs the expression of SV-40 large T-antigen to pancreatic β cells in transgenic mice, and

TABLE 1

Insulin Content of β Cells

Origin	Insulin content‖ Indicated	Insulin content‖ Calculated (ng/million cells)	Ref
Normal pancreas			
20-day-old rat fetus	10pm/3.5 × 1e6 pancreatic cells	600	22
21-day-old rat fetus	182 ng/mg pancreas		23
3-day-old rat pancreas*	1250 ng/mg pancreas ww	35000	24
Adult rat	45–63 ng/islets	45000–63000	25
Adult rat islet	8400 ng/mg		26
Adult rat islet†	30 ng/islets	30000	27
Adult rat isolated differentiated β cells‡		65000	28
after flow cytometry		22150	29
Syrian hamster		11200	30
Adult Chinese hamster†	56300 ng/mg prot	39500	31
Human islet	26–130 ng/μg dry weight	3900–19500	32
β-cell tumors§			
by nicotinamide and streptozotocin	6900–30400 ng/mg ww	7700–34000	33
by radiation	9300 ng/mg ww	10400	16
Hamster insulinoma	2–13 ng/mg prot	0.2–1.4	34
by BK virus in newborn hamster, R1line	8–40 ng/mg prot	0.900–4.5	35
Human adenoma	0.4–8000 ng/mg ww	0.5–9000	36

* Insulin measured as in ref. 21. Volume of β cells by ultrastructural quantitative analysis represents 5 ± 1.3% of pancreas for weight of 20 mg.

† Protein content: 0.67 to 0.76 microg/islet, which might be counted 1000 β cells/islet.

‡ Cultured 16 h and filtered in Percoll.

§ Density of β cells might be estimated at 70%–80% of tumor, volume of β cells as at least 1400μ3 (57), and protein concentration at 100 mg/g.

‖ Pure insulin estimated at 24 U/mg.

TABLE 2

Functional Characteristics of Some β-cell Models

Origin	Cells	Passage no.	Doubling time (hr)	Insulin content (ng million cells)	Insulin secretion		Stimuli	Inhibitors	Ref.
					Basal	Stimulated			
					(ng/million cells)				
Pancreas explant, 3-day-old rat				35000	110	440–2200	Glucose (11 mM)-cAMP		37
Hamster islet monolayer culture				13000	660	4600	Glucose (7.5 mM)		18, 31
NEDH rat islet tumors (RIN)	RINm		48–76	16–333	6.-83				4
Transplanted in nude mice (m)	RINm5F*	80	48	191	15	34	Glyceraldehyde, IBMX	Adrenaline	38
Directly from rat islet tumor	INS	100	110	6000–12000	80	160–240	Glucose 11.2 mM	IL-1	39–40
SV-40 transformed-islet cells	HIT-5B5	37	33–34	11.6	50	100	Glucose 1.25–7 mM, IBMX	Dexamethasone	18, 41
Syrian hamster	HIT-T15†	39–41		296		13	Glucose 3–10 mM	Somatostatin	
Transgenic mouse β-cell lineages‡							Glucose 7.5–10 mM		
B6 × D2F2	βTC-1	35–75		111	3.8	20	Glucose, AMPc	IFNg or IL-1	19
	βTC-3	10–35	48	600–7300	40	80 (16.7)	Glucose 16.7 mM		19–42
C57BL/6xSJL F2	IgSV195				2.1	2.1	IBMX, glutamine		43
C57BL/6 mouse	MIN 6	15			15	458	Glucose (0.7–25 mM)		44
NOD/Lt	NIT-1	11		1540	80	120	Glucose (0–16.5)		45

* Clone cells.

† Subclone of 5B5.

‡ Total protein concentration per culture well is about 0.240 mg/million cells.

the heritable phenotype of these mice is characterized by the development of islet hyperplasia and insulinoma.

These different cell lines provide simplified models to investigate the growth parameters of β cells at the molecular level and the external signals and oncogene expression that are associated with β-cell mitogenesis. These models also have limitations, since it is not known to what extent their mode of proliferation is representative of normal β-cell growth. However, it might be hypothesized that since β-cell proliferation is a multistep procedure, derived bypasses may thus be used in immortalized or even transformed cells to overcome localized impairment, leading however to integrated control of cellular proliferation and differentiation.

Cell Types and β-Cell Regeneration

Characterization of the Models

Insulin Content in Normal β Cells. Since the main characteristic of normal β cells is to synthesize, stock, and secrete insulin on glucose stimulation, we have reviewed, for the different models used, the insulin content and secretion measured by radioimmunoassay, a method initially developed by Yalow and Berson (1). Table 1 shows the insulin content of normal β cells (22–32) and of various β-cell tumors (33–39). The insulin content of fetal β cells is low, whereas insulin content (23, 46) and biosynthesis (47) in the 3-day-old rat (21) are similar to those observed in adults. Another difference is that fetal β cells poorly respond to glucose, the physiologic stimulus of adult β-cells (7). However, part of the β-cell regulatory system is functional in fetal β cells, as indicated by the insulin secretion induced by cAMP or Ca^{2+}, the positive effect of glucose in the presence of IBMX or forskolin, the ligand-induced stimulation or inhibition of insulin secretion.

Insulin Content and Secretion in Cultured β Cells. Some features of β-cell lines are summarized in Table 2 (4, 18–19, 37–45). The doubling time of cells is generally slow, greater than 24 h, and most of these cells exhibit characteristics similar to those of fetal β cells. These characteristics vary according to the cell line and even within the same line depending on the culture medium and the passage number (18, 41, 48–50). Attempts to obtain an established human β-cell line have so far been unsuccessful (51).

β-Cell Growth Results

A first step in the evaluation of β-cell growth is to determine the serum dependence: serum deprivation is the simplest way to suppress growth factors from the extracellular medium and therefore to determine to what extent cellular growth is dependent on external signals. Cells

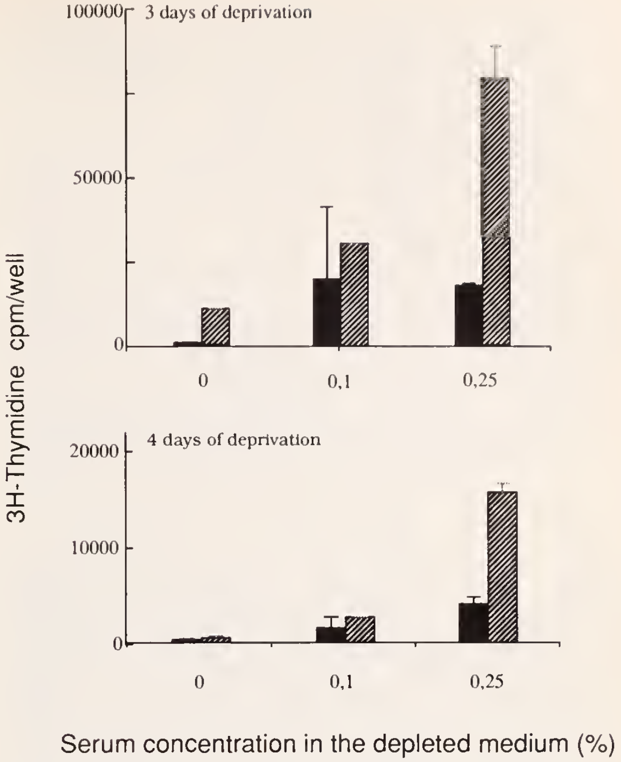


Fig. 1. Arrest of βTC1 cell growth in medium containing low serum concentration. Serum deprivation was allowed to proceed for 3 or 4 days at various serum concentrations, as indicated, in standard DMEM medium. Cells were then submitted to serum stimulation (8% FCS, hatched bars) or medium change (black bars). $[^3H]$ thymidine was added concomitantly for 24 h in each case. Results are means ± SE of triplicate determinations of a representative experiment.

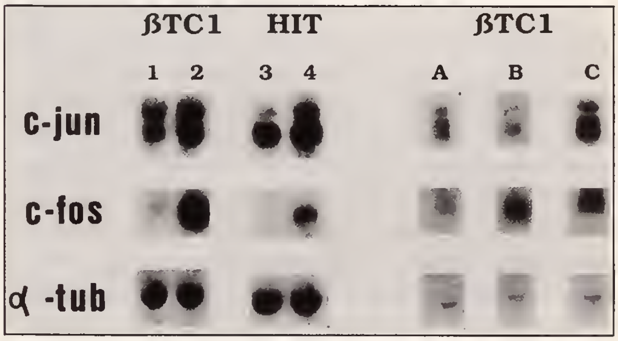


Fig. 2. Expression of cell cycle-related genes. Left Total RNA, 20 μg, was analyzed in Northern blots and hybridized with indicated $[^{32}P]$-labeled probes as follows (lanes 1 to 4): in βTC1, cells were rendered quiescent by 3-day incubation in 0.25% FCS DMEM medium (lane 1) then restimulated for 30 min by addition of 8% FCS (lane 2). In HIT cells, suramin (200μg/mL) was added for 24 h and total RNA was extracted at 0 time (lane 3) or 30 min after suramin removal and no serum was added (lane 4). **Right** total RNA was extracted from βTC1 at quiescence (lane A) and 60 min after a treatment by IBMX (0.5mM, lane B) or forskolin (20μM, lane C). Sizes of mRNAs: c-*fos* 2.2 kb, c-*jun* 2.7 kb and 3.2 kb, and α-tubulin 1.8 kb.

are initially seeded in the presence of serum, facilitating attachment to the dish, and thereafter deprived for one to four days at various serum concentrations. Several methods were used to evaluate the number of arrested cells: TCA-precipitable methyl tritiated thymidine incorporation ([3H] thymidine), histometry and scoring of labeled nuclei, flow cytometric analysis and cytometry of fluorescent bromodeoxyuridine labeling (52). Finally it was checked that arrested cells were still able to resume their cell cycle on the addition of serum or growth factors.

βTC1 Cells. The effect of three or four days of serum deprivation on thymidine incorporation in βTC1 cells (Fig. 1) indicates that the growth of βTC1 cells is serum dependent (53). Optimal conditions for arresting cells at this passage were observed at a minimal serum concentration of 0.25%. We have further determined that cells were synchronized by serum depletion: after a 3-day deprivation, serum was added and [3H] thymidine pulses were applied every three h over 24 h. In these conditions, DNA synthesis increased with time to a maximum at 20 h, whereas only a small incorporation of [3H] thymidine was observed in the serum-deprived control cells. We have also checked that growth was better when glucose was added together with serum (54). In the absence of serum, glucose had also a slight effect on DNA synthesis. Flow cytometric analysis of βTC1 cell cycle indicated 21 h for the G1 phase, 10 h for the S phase, and 12 h for the G2/M phases (53). To study the expression of the cell cycle-related genes, total RNA was extracted 30 min after restimulation with serum (Fig. 2, lane 1 and 2). A strong and fast induction of c-*fos* gene expression occurred in serum-treated cells. Expression of c-*jun* was also increased. The levels of c-*fos* mRNA decreased thereafter. As c-*fos* together with c-*jun* encode transactivating DNA-binding proteins that form nuclear complexes which recognize the AP-1 consensus sequence (55), it can be postulated that the transcription of AP-1-responsive genes constitutes an important step in the progression of βTC1 cells toward DNA synthesis. In quiescent cells, c-*jun* is constitutively expressed. This is likely to be due to the presence of SV-40 large T antigen under the control of the rat insulin II promotor. This also means that the constitutive expression of this gene does not suppress the serum-dependence of this β cell line.

HIT Cells. Serum deprivation for three days, in diverse conditions, did not suppress [3H] thymidine incorporation (56). Even in the absence of serum, DNA synthesis reached values at least as high as those obtained after restimulation with serum. Furthermore, when cells were seeded at increasing concentrations, the incorporation of [3H] thymidine per million cells was greater than what could have been expected according to cell density. These two characteristics fit well with the hypothesis that HIT cells are able to produce growth factors and that they function in an autocrine manner. Similar results were observed using the βTC3 cell line (57), although those cells are less resistant than HIT cells (Fig. 3).

In the experimental design we have followed to investigate the existence of an autocrine regulation of growth, the cells were treated with the polyanionic drug suramin, which has a number of biological effects and particularly prevents the binding of ligands and therefore of growth factors to their cell surface receptors. We have observed that when these conditions were applied to HIT or βTC3 cells, the incorporation of [3H] thymidine was suppressed (Fig. 4). This inhibition was not due to a toxic effect of suramin, since [3H] thymidine incorporation was recovered after suramin removal either in the absence or in the presence of serum (Fig. 4). Furthermore, histoautoradiographic studies in HIT cells demonstrated that the majority of the cells had recovered since most of the nuclei were heavily labeled. Finally, the early responsive genes c-*fos* and c-*jun* are expressed in HIT cells 30 min after suramin removal and in the absence of serum (Fig. 2, lane 3 and 4). This pattern, as well as the constitutive expression of c-*jun,* was also observed in the βTC1 cell line on serum stimulation. In HIT cells the induction is likely to be due to the autocrine secretion of growth factors in the medium, since these early responsive genes are induced in the absence of external growth factors. Finally, the medium conditioned by the HIT cells is able to promote the growth of other cells. Indeed, this conditioned medium is efficient on βTC1-cell DNA synthesis (Fig. 4B).

Two problems remain primary: the role of the SV40 large T antigen in serum independence and the nature of the factors secreted by HIT cells. It could be hypothesized that independence of serum is associated with immortalization induced by large T. However, it has been shown that in polyoma large T or SV-40 large T-immortalized fibroblasts, serum is still required for the activation of transcription factor AP-1, whereas transforming oncogenes such as c-Ha-*ras,* v-*src,* and polyoma middle T abrogate this requirement (58). It could thus be suggested that the serum independence of HIT cells is due to specific factors whose expression does not directly depend on SV-40. Experiments so far have shown that this medium cannot be replaced by any known growth factors: PDGF, IGF-1, EGF, or growth hormone. Therefore, we are now in the process of further characterizing the factor(s) which are secreted by HIT cells and which stimulate the growth of βTC1 cells.

INS-1 Cells. Using another cell line also sensitive to serum deprivation, the INS-1 cell line developed by Asfari, it was possible to observe an effect of growth hormone at the concentration of about 100ng/mL (39). This effect was due to the interaction with a prolactin subtype growth hormone receptor, since as shown in Fig. 5, there is no additive effect of prolactin and growth hormone on INS-1 cell DNA synthesis. The hGH-induced proliferative effect in INS-1 cells appears to be due to a direct action of the hormone: it is not mediated by IGF-1, since IGF-1 is not expressed in these cells, in contrast to what was observed in rat (15, 59–60) or human (61) fetus. However, IGF-2 is expressed, as in fetal cells but its involvement with growth-inducing external signal is still unclear. Neither prolactin nor growth hormone is as efficient as serum for stimulating DNA synthesis in INS-1 cells, and neither substance exerts its effect by increasing c-*fos*, c-*jun*, or cdc2 in this cell line (data not shown). The glucose transporter Glut-2, a specific component of the glucose-sensing system in functional β cells, is highly expressed in the INS-1 cell line (data not shown). Human GH also stimulates DNA synthesis in RIN cells (62), whereas its effect in increasing insulin biosynthesis was only observed after the introduction of exogenous growth hormone receptors (63).

Effect of cAMP. Further studies on the system responsible for the regulation of β-cell growth were performed in βTC1 cells. In this cell line, the dose-dependent rise in the intracellular cAMP levels was correlated with the inhibition of DNA synthesis (64). Forskolin (FK), which generates a higher level of cAMP, is less efficient than IBMX for blocking DNA synthesis (Table 3). Gene expression also differs depending on the drug used (Fig. 2: A, B, and C). The c-*fos* mRNA increased on the addition of IBMX or forskolin (lane B and C), in agreement with the presence of a CRE in the promoter of this gene. The c-*jun* mRNA was enhanced only with forskolin (lane C). Thus the different effects of IBMX and FK on DNA synthesis suggest that the cAMP effect on growth may involve different pathways.

Induction of c-*fos* occurs in response to many agents and has also been observed after interleukin-1 treatment of β cells (65). The role of c-*fos* in β-cell function is not known. Taken together, our data indicate that the resumption of the β-cell division cycle is not the consequence of the induction of c-*fos* itself but requires simultaneous expression of both c-*fos* and c-*jun*, in agreement with results described in other cell lines (55, 66).

TABLE 3

Effect of IBMX or Forskolin on cAMP Content and Incorporation of ^{3}H Thymidine by βTC1 Cells

	Drug concentration (mM)	[3H] Thymidine incorporation (cpm/well)	cAMP (picomoles/well)
Serum		94000	0.2
with IBMX	.05	59000	
	.5	9000	1.2
	2	400	
with forskolin	0.02	36000	4

Cells were rendered quiescent by 3-day incubation in serum-free medium. Medium was then replaced by 8% fetal calf serum Dulbecco's modified minimum essential medium. Drugs were added simultaneously with serum. [3H] Thymidine is applied for 24 h stimulation. For cAMP determination, quiescent βTC1 cells in 24 wells were incubated 1 h with serum in absence or presence of drug as indicated. After lysis in HClO4, intracellular cAMP was determined by radioimmunoassay.

Studies with other protooncogenes (data not shown) indicated that in both βTC1 and HIT cells, the level of c-*myc* was low or even undetectable. However, glucose-induced stimulation of both c-myc expression and cell proliferation was observed in RIN cells (67), whereas sodium dibutyrate was shown to inhibit cellular proliferation and decreased levels of c-*myc* (68). We have also been unable to detect the reg gene found in regenerating islets after pancreatectomy but not in normal islets (69).

Origin of β Cells

The origin of β cells is not clear, and most authors agree that β cells originate from ductular cells (22). However, the process of their differentiation into functional β cells is not known. We will see shortly our attempt to further characterize in situ the cell types involved at the onset of β-cell regeneration. For that purpose we have chosen streptozotocin-induced diabetes on neonate rats (Portha's model, 23). After injection of streptozotocin at birth, there is destruction of β cells, followed by decrease in insulin content and a blood glucose peak 3–4 days after birth. The insulin content is thereafter partly recovered and the diabetes regresses. The highest rate of recovery takes place in the first week following the peak in blood glucose. We have taken pancreas at the onset of diabetes recovery, 3 days after STZ administration and 24 h after thymidine injection and performed ultrastructural morphometric (70)

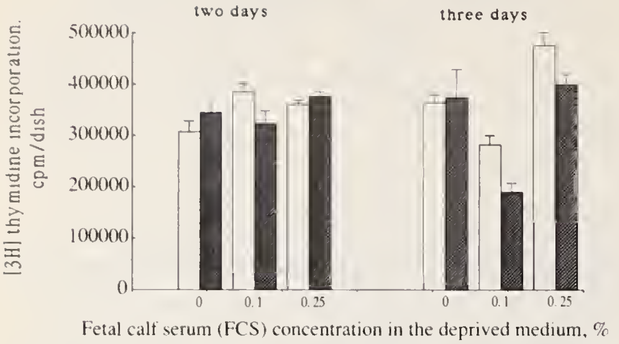


Fig. 3. Effect of serum-free medium on growth of βTC3 cells. Cells were seeded into 24-well costar plates at 90000 cells/well/1 mL of DMEM containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 8% heat-inactivated fetal calf serum. After 24 h incubation cells were washed twice with 2 mL of serum-free DMEM and incubated in this medium for 24 h (left) or 48 h (right). At end of this period, deprivation medium was replaced with DMEM without (clear bars) or with (hatched bars) 8% serum in presence of 1 μCi/mL [3H] thymidine for 24 h. Thymidine incorporation was arrested by addition of 3 drops of 1M ascorbic acid. Cultures were washed twice with cold phosphate-buffered saline and two times with cold 5% trichloroacetic acid. DNA was dissolved in 0.3 mL of 0.1M NaOH, and 0.25 mL of solubilized DNA were transferred into 5 mL of aqualyte scintillation liquid and counted in a LKB scintillation counter. Results are means of triplicate cultures ± SEM.

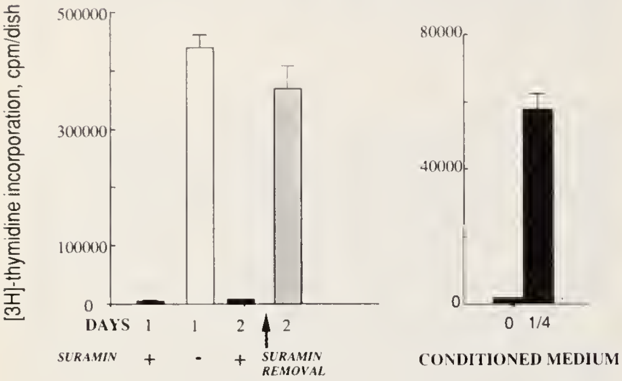


Fig. 4. Autocrine regulation of HIT cells. Left Effect of suramin on [3H] thymidine incorporation by HIT cells. 24 h after plating in 8% fetal calf serum, cells were washed twice with serum and suramin was added for 1 or 2 days (black bars); controls with medium, no added suramin, white bar. Effect of suramin removal in absence of serum, gray bar. Right Quiescent (0 serum) βTC1 cells exposed to HIT-conditioned medium (¼ dilution).

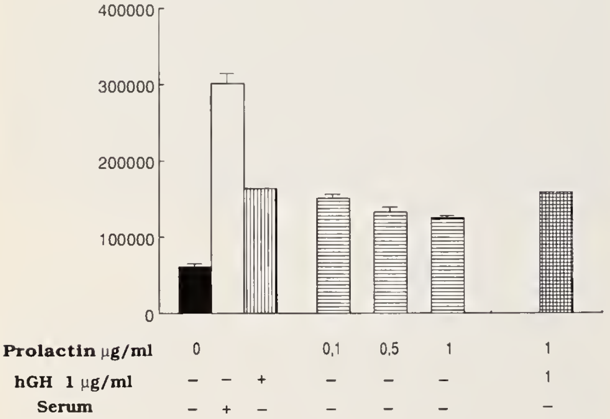
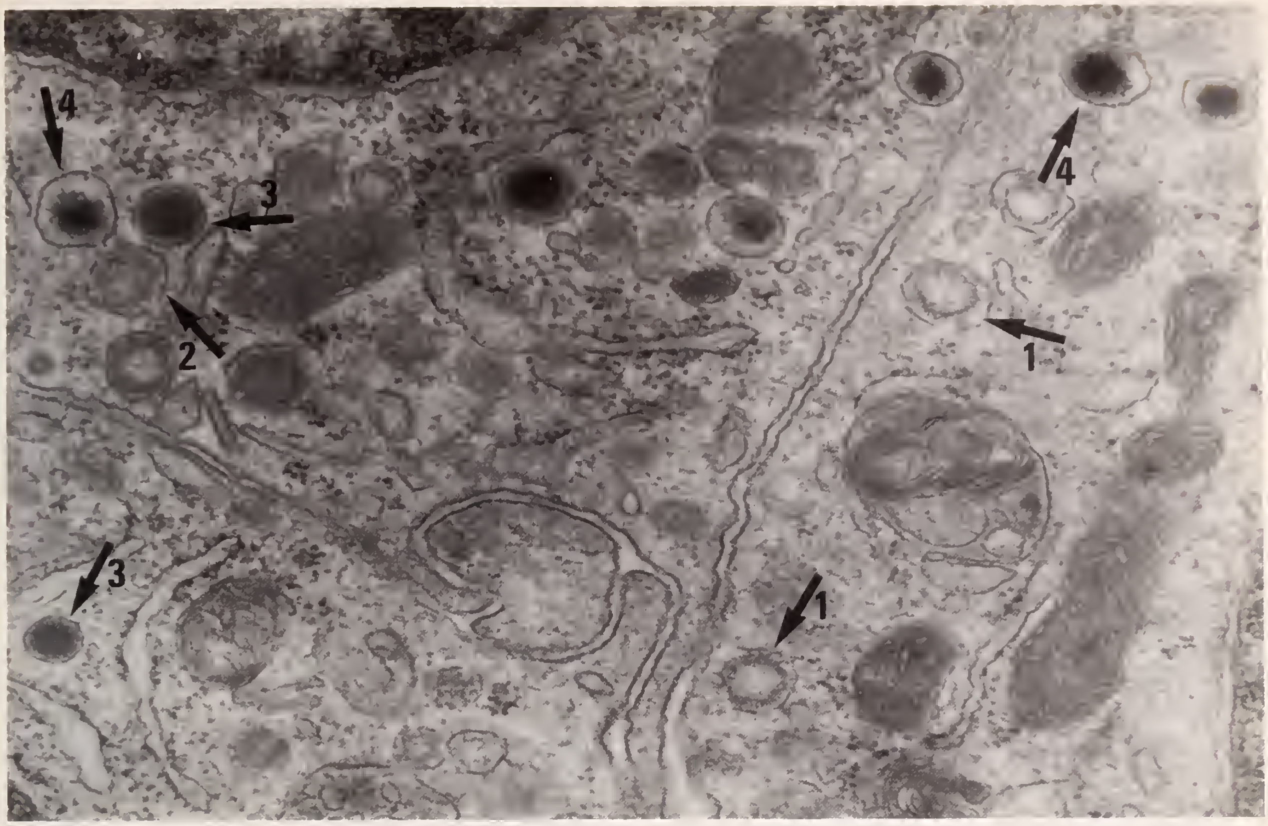


Fig. 5. Effects of prolactin and hGH on INS-1 cell DNA synthesis. Cells were seeded into 24-well costar plates at 0.2 million cells/well/05. mL of RPMI/1640 medium containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated fetal calf serum (HI-FCS). Cultures were incubated for 48 h, then washed twice with 2 mL of serum-free RPMI/1640 medium and incubated in this medium for 100–110 h. Deprivation medium was then replaced with 1 mL of RPMI/1640 medium containing different concentrations (+): either 10% serum, or hGH, or prolactin at three concentrations, in presence of 1 μCi/mL [3H] thymidine for 24 h. Thymidine incorporation performed as indicated for Fig. 3. Experiment is representative of 2 similar experiments; results are means of triplicate cultures ± SEM.

and immunochemical (71) quantitative studies. [3H] thymidine was injected 1 h before sampling of the pancreas to determine the cells which were involved in the β cell regeneration.

Based on ultrastructural criteria, we have called polymorphic cells which contain heterogeneous granules (insulin-like, glucagon-like) and numerous vesicular granules the polymorphic endocrine pancreatic cell subtype (PEPS) (Fig. 6). After sequential double gold immunolabeling (Fig. 7), gold particles of large diameter for glucagon and small gold particles for insulin could be detected within the same cell. Furthermore, both types of particles were present in the same structure, indicating that these cells are pluripotent. In addition, quantitative studies on the repartition of PEPS indicate that considerable change takes place depending on the duration of diabetes recovery (Fig. 8). [3H] thymidine incorporation persisted in the PEPS cells, whereas it was practically suppressed in β cells. Increased [3H] thymidine incorporation was observed on ductular cells, either in focus as previously observed (72) or in canalicules of different size. Three days after streptozotocin injection, the proportion of β cells was considerably decreased, whereas the percentage of PEPS was high. After recovery, the β cell percentage increased, whereas that of PEPS de-



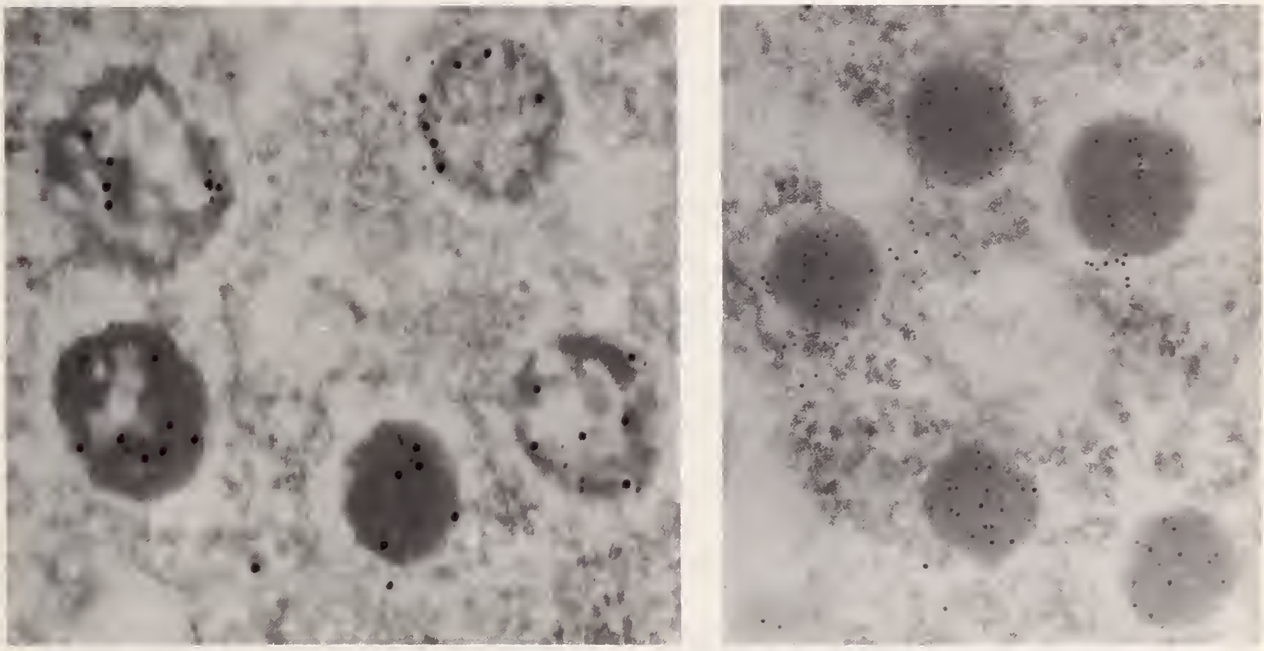


Fig. 6. Top Ultrastructural characteristics of polymorphic endocrine pancreatic cell subtype (*PEPS*) at onset of β-cell regeneration following streptozotocin-induced diabetes of neonates (Portha's model). Representative high-resolution micrograph of PEPS cells containing polymorphic granules termed vesicular (1), semidense (2), dense (3), and insulin-like (4); formaldehyde-glutaraldehyde and osmium fixed. Lead-citrate and uranylacetate stained, original magnification ×60000. **Fig. 7. Bottom** Immunocharacterization of granules of PEPS. **Left**, glucagon and insulin distribution was determined using indirect sequential double immunogold staining. Colocalization of antiglucagon antibody (gold particles/10 nm) with antiinsulin antibody (gold particles 5 nm) is seen in semidense and dense granules. In β-like granules (**right**) only presence of insulin is detected (original magnification ×120.000). Granules of same type as in Fig. A. However, for immunocytochemistry, osmium fixation was omitted, and grids were double-faced etched with H202.

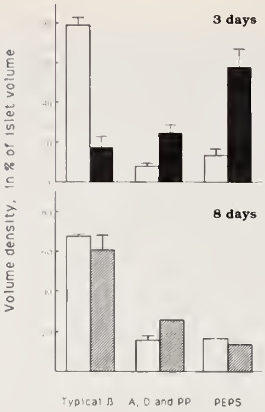


Fig. 8. Repartition of endocrine cells in islet during recovery of diabetes. Volume density of each endocrine cell subtype, measured by point counting method of Webel (total number of counted points = x), expressed as % ± SEM of total islet volume in streptozotocin-treated rats 3 days (above), n = 3, and 8 days (below), n = 3, after injection of buffer or drug for controls (gray bars) and diabetics (hatched bars).

creased. Therefore PEPS are insulin-secreting pluripotent cells. They are resistant to streptozotocin and are able to divide, as shown by [3H] thymidine incorporation. They may represent an intermediary cell subtype which, by further differentiation, could have participated in the development of true β cells and recovery from diabetes.

Discussion and Conclusions

Several pathways are responsible for the development of the β cell. The differentiation of a preexisting pool of cells is suggested by the presence of pluripotent polymorphic endocrine pancreatic cell subtype (PEPS) which occurs at the onset of β-cell regeneration and which was observed in situ following streptozotocin-induced destruction of fully differentiated β cells. It accounts at least partly for the recovery from this form of neonatal diabetes.

The proliferation of differentiated β cells is another possibility. Our results on the β-cell lines suggest that they have retained cell-specific growth regulatory elements. Although little is known of the pathways by which SV-40 determines cell transformation, it is unlikely that SV-40 could mediate the simultaneous induction of c-*fos* and c-*jun*. It has rather been shown that SV-40 could induce a secretion of PDGF-like substances (73–78) and in some instances a constitutive expression of *jun*. Other data suggest that T antigen could also act by making cells more sensitive to growth factors. PDGF, which binds to a tyrosine kinase receptor and can also activate phospholipase C, is known to induce the state of competence in fibroblasts. The addition of PDGF to fetal rat islets (80) or the coexpression of the PDGF B chain and the PDGF β receptor (81) in isolated pancreatic islet cells induces a twofold increase of islet DNA synthesis. However, PDGF does not account for the stimulatory effect of serum or HIT-conditioned serum on the βTC1 cell. Other factors are responsible for the regulation of β-cell growth, since we have shown that the reentry of βTC1 into the cycle was not dependent on PDGF, whereas platelet-poor plasma was already strongly mitogenic (53). Taken together, those data suggest that PDGF could be necessary for initiating the G0/G1 transition in normal β cells and that this step is bypassed in cells expressing the large T antigen of SV-40.

Thus it appears that the system which couples external signals to the early expression of the c-*fos* and c-*jun* genes is retained in β cells and is not the consequence of immortalization of the cells. This indicates that the ultimate formation of specific protein-DNA complex in both these cell lines will stimulate the AP-1 responsive genes and therefore determine cell cycle progression.

The external signal responsible for the resumption of the β-cell cycle and the membrane receptors involved in the transduction of this growth signal are still not known. It was thus of interest to find the presence of this β-cell growth factor(s) in serum and in HIT-conditioned medium. Furthermore, we have shown that when the cAMP pathway was activated, it inhibited rather than stimulated the growth of βTC1 cells. Studies on other β-cell lines such as INS-1 and RIN cells showed that they have retained a specific proliferative response to growth hormone which is not detectable in βTC1 or HIT cells. These features of the β-cell lines are likely to render them useful for studying the molecular events implicated in the distinct pathways regulating β-cell growth.

References

1. Yalow RS, Berson SA. Immuno-assay of endogenous plasma insulin in man. J Clin Invest 1960; 39:1157–1175.
2. Rosselin G. Physiological relevance of receptor characterization in the study of entero-insular axis. In: Blasquez F, ed. Frontiers of hormone research. Basel: Karger 1987, 51–82.
3. Chick L, Boston MD. Beta cell replication in rat panceatic monolayer cultures effect of glucose, tolbutamide, glucocorticoid, growth hormone and glucagon. Diabetes 1973; 22(9):687–693.
4. Gazdar AF, Chick WL, Oie HK, Sims HL, King DL, Weir GC, Lauris V. Continuous, clonal, insulin- and somatostatin-secreting cell lines established from a transplantable rat islet cell tumor. Proc Natl Acad Sci USA 1980; 77(6):3519–3523.
5. Logothetopoulos J. Islet cell regeneration and neogenesis. In: Steiner DF, Freinkel N, eds. Handbook of physiology, Sect 7: endocrinology, vol 1, 67–76, Baltimore: Williams & Wilkins, 1972.
6. Swenne I, Bone AJ, Howell SI, Hellerström C. Effects of glucose and amino acids on the biosynthesis of DNA

and insulin in fetal rat islets maintained in tissue culture. Diabetes 1980; 29:686–776.

7. Hellerström C. Growth pattern of pancreatic islets in animals. In: Vollk BW et al., eds. The diabetic pancreas, 2nd ed. New York: Plenum 1984, 53–79.
8. Bourne HR, Rozengurt E. An 18,000 molecular weight polypeptide induces early events and stimulates DNA synthesis in cultured cells. Proc Natl Acad Sci USA 1976; 73:4555–4559.
9. Pardee AB, Campisi J, Gray HE, Dean M, Sonenshein G. Cellular oncogenes, growth factors and cellular growth control. In: Mediators in cell growth and differentiation. New York: Raven Press, 1985.
10. Bonner-Weir S, Deery D, Leathy JL, Weir GC. Compensatory growth of pancreatic β-cells in adult rats after short-term glucose infusion. Diabetes 1989; 38:49–53.
11. Lehy T, Puccio F. Promoting effect of bombesin on the cell proliferation in the rat endocrine pancreas during the early postnatal period. Regulatory Peptides 1990; 27:87–96.
12. Komyia I, Baetens D, Kuwajima M, Orci L, Unger RH. Compensatory capabilities of islets of BB/wor rats exposed to sustained hyperglycemia. Metabolism 1990; 39(6):614–618.
13. Swenne I. The role of glucose in the in vitro regulation of cell cycle kinetics and proliferation of fetal pancreatic β-cell. Diabetes 1982; 31:754–760.
14. Swenne I, Hill DJ. Growth hormone regulation of DNA replication, but not insulin production, is partly mediated by somatomedin-C/insulin-like growth factor in isolated pancreatic islets from adult rats. Diabetologia 1989; 32:191–197.
15. Kirkman HA. Stanford Univ Med Bull 1962; 20:163–166.
16. Chick WL, Warren S, Chute RN, Like AA, Lauris V, Kitchen KC. A transplantable insulinoma in the rat. Proc Natl Acad Sci USA 1977; 74:628–632.
17. Bhathena SJ, Oie HK, Gazdar AF, Voyles NR, Wilkins SD, Recant L. Insulin, glucagon and somatostatin receptors on cultured cells and clones from rat islet tumor. Diabetes 1982; 31:521–531.
18. Santerre RF, Cook RA, Crisel RMD, Sharp JD, Schmidt RJ, Williams DC, Wilson CP. Insulin synthesis in a clonal cell line of simian virus 40-transformed hamster pancreatic beta cells. Proc Natl Acad Sci USA 1981; 78(7):4339–4343.
19. Efrat S, Linde S, Kofod H, et al. Beta cell lines derived from transgenic mice expressing a hybrid insulin gene-oncogene. Proc Natl Acad Sci USA 1988; 85:9037–9041.
20. Hanahan D. Transgenic mice as probes into complex systems. Science 1989; 246:1265–1275.
21. Rosselin G, Assan R, Yalow RS, Berson SA. Separation of antibody-bound and unbound peptide hormones labelled with iodine-131 by talcum powder and precipitated silica. Nature (Lond) 1966; 212:355–357.
22. Pictet R, Rutter WJ. Development of the embryonic endocrine pancreas. In: Steiner DF, Freinkel N, eds. Handbook of physiology, Sect 7: endocrinology, vol 1, 26–66. Baltimore: Williams & Wilkins, 1972.
23. Portha B, Levacher C, Picon L, Rosselin G. Diabetogenic effect of streptozotocin in the rat during the perinatal period. J Am Diabetes Assoc 1974; 23(11):889–895.
24. Jarousse CL, Rançon F, Rosselin G. Hormogenèse périnatale de l'insuline et du glucagon chez le rat. CR Acad Sci Paris 1973; 276:585–588.
25. Trimble ER, Renold AE. Ventral and dorsal areas of rat pancreas: islet hormone content and secretion. Am Physiolog Soc 1981; E422–E427.
26. Dixit PK, Morgan CR, Lindall AW Jr, Lazarow A. Proc Soc Exp Biol Med 1969; 131:1124.
27. Halban PA, Wollheim CB, Blondel B, Meda P, Niesor EN, Mintz DH. The possible importance of contact between pancreatic islet cells for the control of insulin release. Endocrinology 1982; 111(1):86–94.
28. Pipeleers D, Veld PA, Maes E, Van De Winkel M. Glucose induced insulin release depends on functional cooperation between islet cells. Proc Natl Acad Sci USA 1982; 79:7322–7325.
29. Fletcher DJ, McLean Grogan W, Barras E, Weir GC. Hormone release by islet β-cell enriched and A and D cell enriched populations prepared by flow cytometry. Endocrinology 1983; 113(5):1791–1798.
30. Fong HK, Chick WL, Sato GH. Hormones and factors that stimulate growth of a rat islet tumor cell line in serum-free medium. Diabetes 1981; 30:1022–1028.
31. Siegel EG, Wolheim CB, Sharp GWG, Herberg L, Renold AE. Defective calcium handling and insulin release in islets from diabetic Chinese hamsters. Biochem J 1979; 180:233–236.
32. Gepts W. Endocrine pancreas. In: Steiner DF, Freinkel N, eds. Handbook of physiology. Sect 7, vol 1. Baltimore: Williams & Wilkins, 1972, 289.
33. Dixit PK, Bauer GE. Studies on rats with islet beta cell tumors induced by nicotinamide and streptozotocin (39368). Proc Soc Exp Biol Med 1976; 152:232–236.
34. Rae PA, Yip CC, Shimmer BP. Can J Physiol Pharmacol 1979; 57:819–824.
35. Uchida S, Watanabe S, Alzawa T, Furuno A, Muto T. Polyoncogenicity and insulinoma-inducing ability of BK virus, a human papovavirus, in Syrian golden hamsters. J Natl Cancer Inst 1979; 63:119–126.
36. Creutzfeldt S, Arnold R, Creutzfeld C, Deuticke U, Frerichs H, Track NS. Biochemical and morphological investigations of 30 human insulinomas: correlation between the tumour content of insulin and proinsulin-like components and the histological and ultrastructural appearance. Diabetologia 1973; 9:217.
37. Jarousse CL, Rosselin G. Interaction of amino acids and cyclic AMP on the release of insulin and glucagon by newborn rat pancreas. Endocrinology 1975; 96(1):168–177.
38. Praz GA, Halban PA, Wollheim CB, Blondel B, Strauss AJ, Renold AE. Regulation of immunoreactive-insulin release from a rat cell line (RINm5F). Biochem J 1983; 210:345–352.
39. Asfari M, Bréant B, Rosselin G. Induction of DNA synthesis and gene expression by human growth hormone in a highly differentiated rat insulinoma cell culture. Diabetes (suppl 1) 1991; 857:215A.
40. Asfari M, Janjic D, Meda P, Li G, Halban PA, Wolheim CB. Establishment of 2-mercaptoethanol dependent differentiated insulin secreting cell lines. Endocrinology 1992; 130:167–170.
41. Zhang H-J, Walseth TF, Robertson RP. Insulin secretion and cAMP metabolism in HIT cells: reciprocal and serial passage-dependent relationships. Diabetes 1989; 38:44–48.
42. D'Ambra R, Surana M, Efrat S, Starr RG, Fleisher N. Regulation of insulin secretion from β-cell lines derived from transgenic mice insulinomas resembles that of normal β-cells. Endocrinology 1990; 126:2815–2822.
43. Gilligan A, Jewett L, Simon D, et al. Functional pancreatic β-cell line from SV40 T-antigen transgenic mouse. Diabetes 1989; 38:1056–1062.

44. Miyazaki J-I, Araki K, Yamato E, et al. Establishment of a pancreatic β-cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoform. Endocrinology 1990; 127(1):126–132.

45. Hamaguchi K, Gaskins HR, Leiter E. NIT-1, a pancreatic β-cell line established from a transgenic NOD/lt mouse. Diabetes 1991; 40:842–849.

46. Pictet RL, Rall LB, Phelps P, Rutter WJ. The neural crest and the origin of the insulin-producing and other gastrointestinal hormone-producing cells. Science 1976; 191:191–192.

47. Garcia SD, Jarousse C, Rosselin G. Biosynthesis of proinsulin and insulin in newborn rat pancreas: interaction of glucose, cyclic AMP, somatostatin and sulfonylureas on the [3H] leucine incorporation into immunoreactive insulin. J Clin Invest 1976; 57:230–243.

48. Richardson SB, Eyler N, Twente S, Monaco M, Altzsuler N, Gibson M. Effects of vasopressin on insulin secretion and inositol phosphate production in a hamster beta cell line (HIT). Endocrinology 1990; 126(2):1047–1052.

49. Regazzi R, Li G, Deshusses J, Wolheim CB. Stimulus-response coupling in insulin-secreting HIT cells: effect of secretagogues on cytosolic ca2+, diacylglycerol and protein kinase C activity. J Biol Chem 1990; 265(25): 15003.

50. Schubart UK, Fleischer N, Erlichman J. Ca2+-dependent protein phosphorylation and insulin release in intact hamster insulinoma cells. J Biol Chem 1980; 255(23): 11063–11066.

51. Soldevila G, Buscema M, Marini V, et al. Transfection with SV40 gene of human pancreatic endocrine cells. J Autoimmunity 1991; 4:381–396.

52. Terry NHA, White RA, Meistrich ML, Calkins DP. Evaluation of flow cytometric methods for determining population potential doubling times using cultured cells. Cytometry 1991; 12:234–241.

53. Breant B, Lavergne C, Rosselin G. Cell cycle and gene expression in the insulin producing pancreatic cell line βTC1. Diabetologia 1990; 33:586–592.

54. Breant B, Lavergne C, Rosselin G. Glucose stimulates DNA synthesis in quiescent βTC1 cells. Diabetologia 1989; 32(7):61, 470A.

55. Vogt PK, Tjian R. Jun: a transcriptional regulator turned oncogenic. Oncogene 1988; 3:3–7.

56. Breant B, Lieuvin A, Lavergne C, Rosselin G. Autocrine secretion of growth factors by HIT T15 β cells: regulation of insulin and cell cycle-related gene expression. Diabetes (suppl 1): 1991; 93A, 372.

57. Dean PM. Ultrastructural morphometry of the pancreatic β-cell. Diabetologia 1973; 9:115–119.

58. Wasylyk C, Imler JL, Wasylyk B. Transforming but not immortalizing oncogenes activate the transcription factor PEA1. EMBO J 1988; 7(8):2475–2483.

59. Romanus JA, Rabinovitch A, Rechler MM. Neonatal rat islet cell cultures synthesize insulin-like growth factor I. Diabetes 1985; 34:696.

60. Smith FE, Rosen KM, Villa-Komaroff L, Weir GC, Bonner-Weir S. Enhanced insulin-like growth factor I gene expression in regenerating rat pancreas. Proc Natl Acad Sci USA 1991; 88:6152–6156.

61. Scharfmann R, Corvol M, Czernichow P. Characterization of insulinlike growth factor I produced by fetal rat pancreatic islets. Diabetes 1989; 38(6):686–690.

62. Petersen DE, Billestrup N, Hoiriis NJ. Effect of growth hormone and serum on the expression of the proto-oncogenes c-jun and c-fos in insulin producing cells. Biomed Biochim Acta 1990; 49(12):1171–1175.

63. Billestrup N, Moldrup A, Serup P, Mathews LS, Norstedt G, Nielsen JH. Introduction of exogenous growth hormone receptors augments growth hormone-responsive insulin biosynthesis in rat insulinoma cells. Proc Natl Acad Sci USA 1990; 871(8):7210–7214.

64. Lavergne C, Bréant B, Rosselin G. Effects of agents modulating insulin secretion on βTC1 cell proliferation, insulin and cell-cycle related gene expression. Diabetes 1991; 373(suppl 1):94A.

65. Hughes JH, Watson MA, Easom RA, Turk J, McDaniel ML. Interleukin-1 induces rapid and transient expression of the c-fos protooncogene in isolated pancreatic islets and in purified β-cells. FEBS Lett 1990; 266:1,2,33–36.

66. Buchou T, Mester J. Fibroblast growth factor-dependent mitogenic signal transduction pathway in chemically transformed mouse fibroblasts is similar to but distinct from that initiated by phorbol esters. J Cell Physiol 1990; 142:559–565.

67. Yamashita S, Tobinaga T, Ashizawa K, Nagayama Y, Koyota A, Harakawa S, Inoue S, Hirayu H, Izumi M, Nagataki S. Glucose stimulation of protooncogene expression and deoxyribonucleic acid synthesis in rat islet cell line. Endocrinology 1988; 123(4):1825–1828.

68. Philippe J, Drucker DJ, Chick WL, Habener JF. Transcriptional regulation of genes encoding insulin, glucagon, and angiotensinogen by sodium butyrate in a rat islet cell line. Mol Cell Biol 1987:560–563.

69. Watanabe T, Yonekura H, Terazono K, Yamamoto H, Okamoto H. Complete nucleotide sequence of human reg gene and its expression in normal and tumoral tissues: the reg protein, pancreatic stone protein and pancreatic thread protein are one and the same product of the gene. J Biol Chem 1990; 265(13):7432–7439.

70. Astesano A, Dautriche N, Anteunis A, Rosselin G. Islets: Morphology-differentiation. EASD Diabetologia (abstr) 1990; P383, A108.

71. Ferrand N, Basmaciogullari A, Astesano A, Anteunis A, Rosselin G. Colocalization of immunoreactive hormones in the granules of regenerating pancreatic endocrine cells in reversible diabetes as revealed by ultrastructural double-label immunostaining. Diabetes 1991; 181(suppl 1):46A.

72. Dutrillaux MC, Portha B, Roze C, Hollande E. Ultrastructural study of pancreatic β cell regeneration in newborn rats after destruction of streptozotocin. Virchows Arch (cell pathol) 1982; 39:173–185.

73. Bleiberg I, Harvey AK, Smale G, Grotendorst GR. Identification of a PDGF-like mitoattractant produced by NIH/313 cells after transformation with SV40. J Cell Physiology 1985; 123(2):161–166.

74. Burke RR. Induction of cell proliferation by a migration factor released from a transformed cell line. Exp Cell Res 1976; 101:293–298.

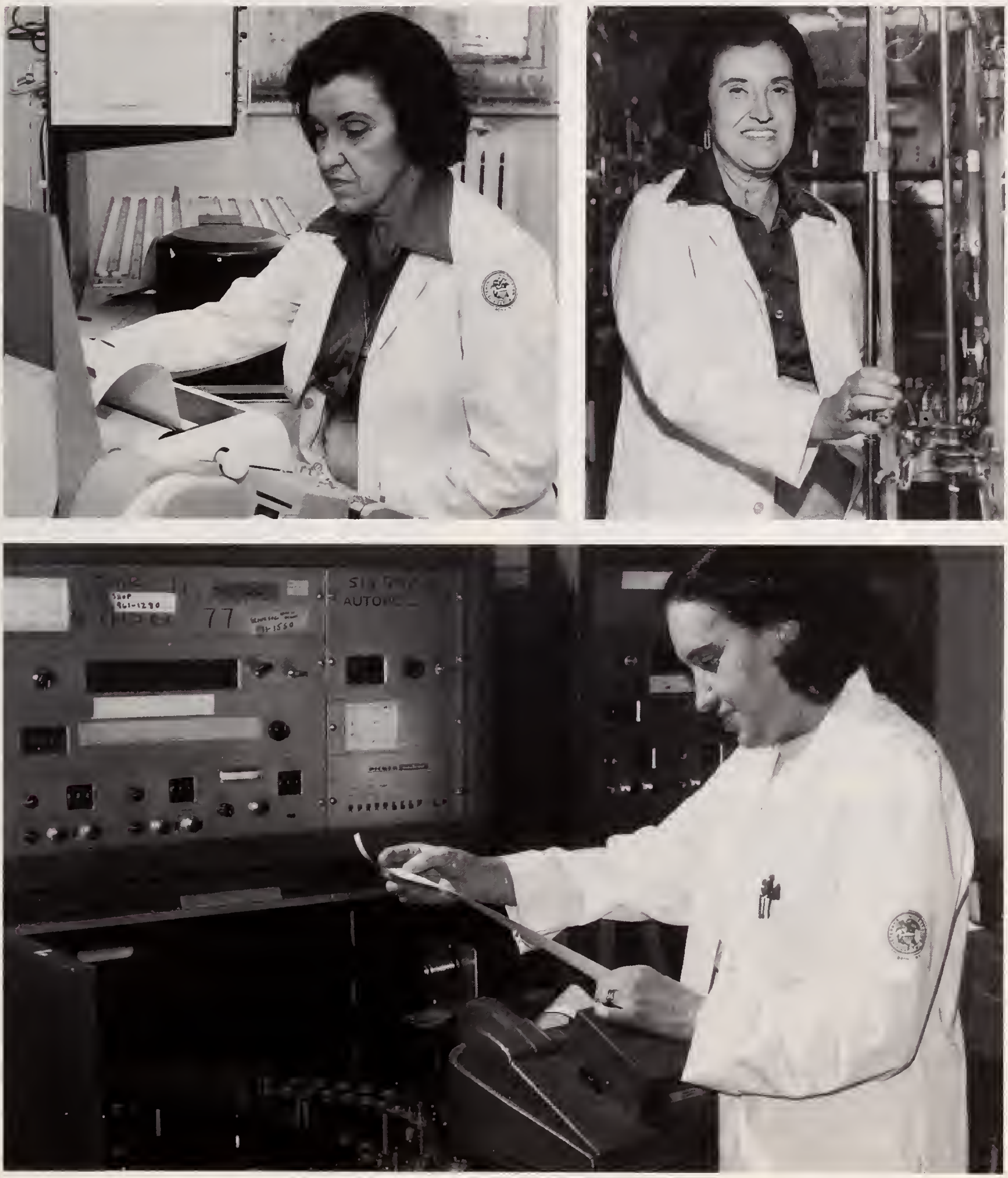
75. Dicker P, Pohanpelto P, Pettican P, Rozengurt E. Similarities between fibroblast-derived growth factor and platelet-derived growth factor. Exp Cell Res 1981; 135;221–227.

76. Estervig DN, Minoo P, Tzen CY, Scott RE. Inhibition of simian virus 40T-antigen expression by cellular differentiation. J Virol 1989; 63:2718–2725.

77. Werner S, Hofscheneider PH, Sturzl M, Dick I, Roth WK. Cytochemical and molecular properties of simian virus 40 transformed Kaposi's sarcoma-derived cells: evi-

dence for the secretion of a member of the fibroblast growth factor family. J Cell Physiol 1989; 141(3):490–502.

78. Piette J, Yaniv M. Two different factors bind to the alpha domain of the polyoma virus enhancer: one of which also interacts with the SV40 and c-fos enhancers. EMBO J 1987; 6(5):1331–1337.
79. Ruderman NR, Kapeller R, White MF, Cantley LC. Activation of phosphatidylinositol 3-kinase by insulin. Proc Natl Acad Sci USA 1990; 87:1411–1415.
80. Swenne I, Heldin CH, Hill DJ, Hellerström C. Effects of platelet-derived growth factor and somatomedin-C/insulin-like growth factor I on the deoxyribonucleic acid replication of fetal rat islets of Langerhans in tissue culture. Endocrinology 1988; 122(1):214–218.
81. Welsh M, Claesson-Welsh L, Hallberg A, et al. Coexpression of the platelet-drived growth factor (PDGF) B chain and the PDGF β receptor in isolated pancreatic islet cells stimulates DNA synthesis. Proc Natl Acad Sci USA 1990; 87:5807–5811.



Rosalyn S. Yalow at work in the laboratory.

A+
NEW INDICATION

ONLY ONE H_2-ANTAGONIST HEALS REFLUX ESOPHAGITIS AT DUODENAL ULCER DOSAGE. ONLY ONE.

Of all the H_2-receptor antagonists, only Axid heals and relieves reflux esophagitis at its standard duodenal ulcer dosage. Axid, **150** mg b.i.d., relieves heartburn in **86%** of patients after one day and **93%** after one week.[1]

AXID®
nizatidine
150 mg b.i.d.

ACID TESTED. PATIENT PROVEN.

1. Data on file, Lilly Research Laboratories. *See accompanying page for prescribing information.* © 1991, ELI LILLY AND COMPANY NZ-2947-B-249304

AXID®

nizatidine capsules

Brief Summary. Consult the package insert for complete prescribing information.

Indications and Usage: 1. *Active duodenal ulcer*—for up to 8 weeks of treatment at a dosage of 300 mg h.s. or 150 mg b.i.d. Most patients heal within 4 weeks.

2. *Maintenance therapy*—for healed duodenal ulcer patients at a dosage of 150 mg h.s. at bedtime. The consequences of therapy with Axid for longer than 1 year are not known.

3. *Gastroesophageal reflux disease (GERD)*—for up to 12 weeks of treatment of endoscopically diagnosed esophagitis, including erosive and ulcerative esophagitis, and associated heartburn at a dosage of 150 mg b.i.d.

Contraindication: Known hypersensitivity to the drug. Because cross sensitivity in this class of compounds has been observed, H_2-receptor antagonists, including Axid, should not be administered to patients with a history of hypersensitivity to other H_2-receptor antagonists.

Precautions: *General*—1. Symptomatic response to nizatidine therapy does not preclude the presence of gastric malignancy

2. Dosage should be reduced in patients with moderate to severe renal insufficiency.

3. In patients with normal renal function and uncomplicated hepatic dysfunction, the disposition of nizatidine is similar to that in normal subjects.

Laboratory Tests—False-positive tests for urobilinogen with Multistix® may occur during therapy.

Drug Interactions—No interactions have been observed with theophylline, chlordiazepoxide, lorazepam, lidocaine, phenytoin, and warfarin. Axid does not inhibit the cytochrome P-450 enzyme system; therefore, drug interactions mediated by inhibition of hepatic metabolism are not expected to occur. In patients given very high doses (3,900 mg) of aspirin daily, increased serum salicylate levels were seen when nizatidine, 150 mg b.i.d., was administered concurrently.

Carcinogenesis, Mutagenesis, Impairment of Fertility—A 2-year oral carcinogenicity study in rats with doses as high as 500 mg/kg/day (about 80 times the recommended daily therapeutic dose) showed no evidence of a carcinogenic effect. There was a dose-related increase in the density of enterochromaffin-like (ECL) cells in the gastric oxyntic mucosa. In a 2-year study in mice, there was no evidence of a carcinogenic effect in male mice, although hyperplastic nodules of the liver were increased in the high-dose males as compared with placebo. Female mice given the high dose of Axid (2,000 mg/kg/day, about 330 times the human dose) showed marginally statistically significant increases in hepatic carcinoma and hepatic nodular hyperplasia with no numerical increase seen in any of the other dose groups.The rate of hepatic carcinoma in the high-dose animals was within the historical control limits seen for the strain of mice used. The female mice were given a dose larger than the maximum tolerated dose, as indicated by excessive (30%) weight decrement as compared with concurrent controls and evidence of mild liver injury (transaminase elevations). The occurrence of a marginal finding at high dose only in animals given an excessive and somewhat hepatotoxic dose, with no evidence of a carcinogenic effect in rats, male mice, and female mice (given up to 360 mg/kg/day, about 60 times the human dose), and a negative mutagenicity battery are not considered evidence of a carcinogenic potential for Axid.

Axid was not mutagenic in a battery of tests performed to evaluate its potential genetic toxicity, including bacterial mutation tests, unscheduled DNA synthesis, sister chromatid exchange, mouse lymphoma assay, chromosome aberration tests, and a micronucleus test.

In a 2-generation, perinatal and postnatal fertility study in rats, doses of nizatidine up to 650 mg/kg/day produced no adverse effects on the reproductive performance of parental animals or their progeny.

Pregnancy—Teratogenic Effects—Pregnancy Category C—Oral reproduction studies in rats at doses up to 300 times the human dose and in Dutch Belted rabbits at doses up to 55 times the human dose revealed no evidence of impaired fertility or teratogenic effect; but, at a dose equivalent to 300 times the human dose, treated rabbits had abortions, decreased number of live fetuses, and depressed fetal weights. On intravenous administration to pregnant New Zealand White rabbits, nizatidine at 20 mg/kg produced cardiac enlargement, coarctation of the aortic arch, and cutaneous edema in 1 fetus, and at 50 mg/kg, it produced ventricular anomaly, distended abdomen, spina bifida, hydrocephaly, and enlarged heart in 1 fetus. There are, however, no adequate and well-controlled studies in pregnant women. It is also not known whether nizatidine can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Nizatidine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers—Studies in lactating women have shown that 0.1% of an oral dose is secreted in human milk in proportion to plasma concentrations. Because of growth depression in pups reared by treated lactating rats, a decision should be made whether to discontinue nursing or the drug, taking into account the importance of the drug to the mother.

Pediatric Use—Safety and effectiveness in children have not been established.

Use in Elderly Patients—Healing rates in elderly patients were similar to those in younger age groups as were the rates of adverse events and laboratory test abnormalities. Age alone may not be an important factor in the disposition of nizatidine. Elderly patients may have reduced renal function.

Adverse Reactions: Worldwide, controlled clinical trials included over 6,000 patients given nizatidine in studies of varying durations. Placebo-controlled trials in the United States and Canada included over 2,600 patients given nizatidine and over 1,700 given placebo. Among the adverse events in these placebo-controlled trials, only anemia (0.2% vs 0%) and urticaria (0.5% vs 0.1%) were significantly more common in the nizatidine group. Of the adverse events that occurred at a frequency of 1% or more, there was no statistically significant difference between Axid and placebo in the incidence of any of these events (see package insert for complete information).

A variety of less common events were also reported; it was not possible to determine whether these were caused by nizatidine.

Hepatic—Hepatocellular injury (elevated liver enzyme tests or alkaline phosphatase) possibly or probably related to nizatidine occurred in some patients. In some cases, there was marked elevation (>500 IU/L) in SGOT or SGPT and, in a single instance, SGPT was >2,000 IU/L. The incidence of elevated liver enzymes overall and elevations of up to 3 times the upper limit of normal, however, did not significantly differ from that in placebo patients. All abnormalities were reversible after discontinuation of Axid. Since market introduction, hepatitis and jaundice have been reported. Rare cases of cholestatic or mixed hepatocellular and cholestatic injury with jaundice have been reported with reversal of the abnormalities after discontinuation of Axid.

Cardiovascular—In clinical pharmacology studies, short episodes of asymptomatic ventricular tachycardia occurred in 2 individuals administered Axid and in 3 untreated subjects.

CNS—Rare cases of reversible mental confusion have been reported.

Endocrine—Clinical pharmacology studies and controlled clinical trials showed no evidence of anti-androgenic activity due to nizatidine. Impotence and decreased libido were reported with similar frequency by patients on nizatidine and those on placebo. Gynecomastia has been reported rarely.

Hematologic—Anemia was reported significantly more frequently in nizatidine than in placebo-treated patients. Fatal thrombocytopenia was reported in a patient treated with nizatidine and another H_2-receptor antagonist. This patient had previously experienced thrombocytopenia while taking other drugs. Rare cases of thrombocytopenic purpura have been reported.

Integumental—Urticaria was reported significantly more frequently in nizatidine- than in placebo-treated patients. Rash and exfoliative dermatitis were also reported.

Hypersensitivity—As with other H_2-receptor antagonists, rare cases of anaphylaxis following nizatidine administration have been reported. Rare episodes of hypersensitivity reactions (eg, bronchospasm, laryngeal edema, rash, and eosinophilia) have been reported.

Other—Hyperuricemia unassociated with gout or nephrolithiasis was reported. Eosinophilia, fever, and nausea related to nizatidine have been reported.

Overdosage: Overdoses of Axid have been reported rarely. If overdosage occurs, activated charcoal, emesis, or lavage should be considered along with clinical monitoring and supportive therapy. The ability of hemodialysis to remove nizatidine from the body has not been conclusively demonstrated; however, due to its large volume of distribution, nizatidine is not expected to be efficiently removed from the body by this method.

PV 2093 AMP [101591]

Additional information available to the profession on request

Lilly

Eli Lilly and Company
Indianapolis, Indiana
46285

NZ-2947-B-249304

© 1991, ELI LILLY AND COMPANY

When you don't know all the medicines your patients are taking, protecting their health is a crap shoot.

Counseling patients about all their medicines improves their odds of getting well and staying well.

EVERYONE WINS WHEN YOU TALK

Send me a *free* Medicine Counseling Kit.

Name

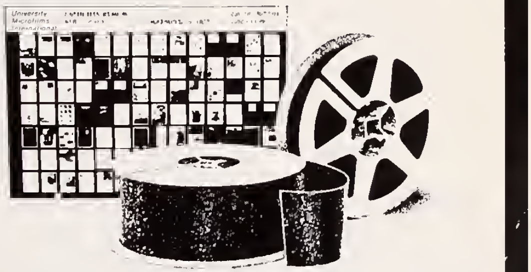
Organization

Street

City State Zip

Mail to:
National Council on Patient Information
and Education
666 Eleventh Street, NW, Suite 810
Washington, DC 20001
To fax your request -- (202) 638-0773

this publication is available in microform



Please send me additional information.

University Microfilms International

300 North Zeeb Road
Dept. P.R.
Ann Arbor, MI 48106
U.S.A.

18 Bedford Row
Dept. P.R.
London, WC1R 4EJ
England

Name ____________________

Institution ____________________

Street ____________________

City ____________________

State ____________________ Zip ________

Instructions for Authors

THE MOUNT SINAI JOURNAL OF MEDICINE, established in 1934 as *The Journal of the Mount Sinai Hospital,* is a peer-reviewed general medical journal. It is indexed or abstracted in *Index Medicus, Current Contents, Science Citation Index, Hospital Literature Index, International Nursing Index, Excerpta Medica, Chemical Abstracts, Biological Abstracts,* and major databases. Theoretical and basic science articles for the clinician, reviews, reports of clinical or research experience, articles on any subject affecting the practice of medicine, short notes in any specialty, book reviews, letters, and articles by medical students are welcome. The *Journal* is a participant in the Agreement on Uniform Requirements for Manuscripts Submitted to Biomedical Journals.

Communications. Address correspondence on manuscripts review to: Managing Editor, *The Mount Sinai Journal of Medicine,* Box 1094, 19 E. 98th Street, 4D, New York, NY 10029-6574. Phone: (212) 241-6108; FAX: (212) 722-6386.

Copyright. The publisher reserves copyright and renewal rights on all published material, and such material may not be published elsewhere without written permission of the publisher. When submitting a manuscript, include the following assignment of copyright in your cover letter: **In consideration of The Mount Sinai Journal of Medicine (MSJM) taking action and reviewing and editing my (our) submission, the author(s) undersigned hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership to The Mount Sinai Journal of Medicine in the event that such work is published by MSJM.** Each author must sign the statement.

Manuscript Preparation. Submit manuscripts in triplicate, including three sets of illustrations. Manuscripts must be typewritten, **double-spaced throughout (including references, legends, and tables),** on one side of 8½ × 11-in. white bond paper, with generous margins. Number pages consecutively. Begin a new page for title page, abstract, first page of text, references, each table, and legends. **Computer-generated** typescripts must be double-spaced and must be easy for editors and typesetters to read. **Computer tapes or disks:** consult managing editor.

Title Page. On the title page, on separate lines (double-spaced), include (1) title of article, phrased as concisely as possible; (2) name of each author, including first name and degree; (3) name and address of departments and institutions from which the work originated; (4) acknowledgments of sources of support; (5) name, place, and date of any conferences at which paper has been delivered; (6) the statement **Address reprint requests to . . . ,** giving full name and address, with zip code, of the appropriate author.

Abstract. Original articles should include an abstract, which should be a single paragraph not exceeding 150 words. State the purposes; basic approach or methods; main findings; principal conclusions. Emphasize new and important aspects. When an abstract is provided, a summary at the end is not necessary. Reviews and other articles without an abstract should conclude with a short summary.

Key Words. Supply 3 to 10 key words or phrases at the bottom of the title page, for use in indexing the article. Use Medical Subject Headings from *Index Medicus.*

Writing Style and Abbreviations. Authors should aim for conciseness and clarity without repetition. Avoid medical jargon, abbreviated phrasing, and obscure or newly coined abbreviations. Define all abbreviations on first use, except those for standard, universally recognized units of measurement and statistical terms. Spell out such terms as white blood cell, hematocrit, superior vena cava.

Units of Measurement. Use SI units, giving range or interval of normal values.

Editing. All papers will be edited to enhance conciseness and clarity without altering meaning and to insure conformity with journal style. The author is responsible for all statements in the article.

Illustrations. Photographs, line drawings, graphs, and charts should increase understanding of the text. Line drawings, graphs, and charts should be professionally prepared. All illustrations should be submitted in triplicate as sharp, high-contrast glossy prints. Photomicrographs must be 5⅛ in. wide. On the back of each print affix a gum label with the number of the figure (numbered consecutively in arabic numerals as cited in text), title of manuscript, name of senior author, and the word "top" with an arrow indicating top edge. Illustrations which are to appear together should be mounted accordingly as plates and should correspond to each other in size. Protect the prints by enclosing them in heavy cardboard; do not use paper clips. Photographs of patients can be published only if a copy of the permission is submitted with the photograph. Eliminate names of patients and hospital numbers from x-rays.

Legends. Legends should not duplicate the text. Double space. Number each legend to match the illustration it accompanies. Illustrations mounted as plates to appear together should be accompanied by a single legend identifying the separate elements by position ("upper left," and so on) or by letters. If letters are used in the legend, a corresponding letter must appear on the print itself. Identify all abbreviations. Indicate magnification and stain for photomicrographs.

Tables. Each table should be typed on a separate page, double spaced. Number tables consecutively as they appear in text, using arabic numerals ("Table 1"). Give each table a brief title (for example, "Effect of DMSO on Rats") and type it on a separate line. If abbreviations or symbols are used in the table, identify them in a footnote.

References. When citing references in your text, the first work cited is numbered 1, and succeeding references are numbered in sequence; enclose the citation number in parentheses and place it before any punctuation: **Cytomegalovirus (1), steroids (2), and recreational drugs (3) have all been implicated (4).** The reference list includes only works cited in the text, numbered in the order in which they are cited. Type, double spaced, following the general arrangement and punctuation style in the examples below (journal title abbreviations conform to *Index Medicus* style):

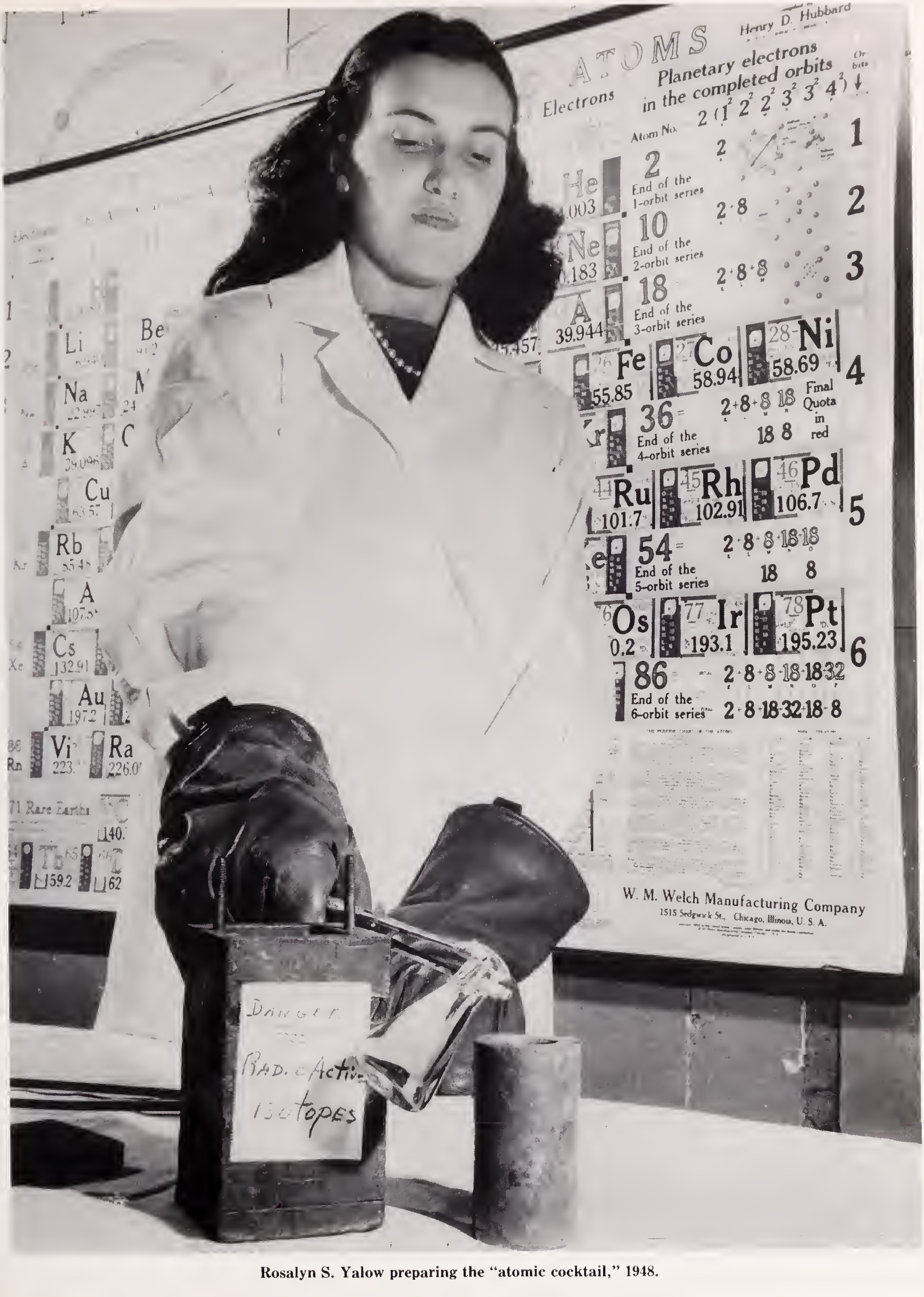
1. **Nadelman RB, Wormser GP. A clinical approach to Lyme disease. Mt Sinai J Med 1990;57:144–156.**

2. **International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. N Engl J Med 1991;324:424–428.**

When a manuscript is accepted for publication, you will be asked to confirm the accuracy of all references.

Proofs. Proofs are sent to the corresponding author from the printer. Stylistic changes which do not alter meaning should not be made at this stage. Because of increased printing charges, the cost of excessive author's alterations beyond routine correction of typographical errors and major errors in data may be billed to the author. Proofs should be corrected and returned to the editorial office within 48 hours.

Reprint Orders. A reprint order form is sent to the corresponding author with proofs; return it to the editorial office.


ATOMS
Henry D. Hubbard
Electrons
Planetary electrons in the completed orbits
Atom No.
End of the 1-orbit series
End of the 2-orbit series
End of the 3-orbit series
End of the 4-orbit series
End of the 5-orbit series
End of the 6-orbit series
Final Quota in red
W. M. Welch Manufacturing Company
1515 Sedgwick St., Chicago, Illinois, U. S. A.
DANGER
RADIOACTIVE ISOTOPES

Rosalyn S. Yalow preparing the "atomic cocktail," 1948.

THE MOUNT SINAI JOURNAL OF MEDICINE

Forthcoming: Grand Rounds

Immunoregulation, Immune Defects, and Clinical Strategies in HIV Infection
H. Clifford Lane, MD
National Institute of Allergy and Infectious Diseases

HIV Infection: Dominant New Face of TB
Lee B. Reichman, MD, MPH
University of Medicine and Dentistry of NJ

Primary Care and HIV Patients
Joseph R. Masci, MD
Mount Sinai School of Medicine

Pulmonary Complications of HIV Infection
Mark Rosen, MD
Mount Sinai School of Medicine

Graves' Disease
Terry Davies, MD
Mount Sinai School of Medicine

Osteoporosis: On the Verge of Rational Effective Therapy?
John Paul Bilezikian, MD
Columbia University College of Physicians and Surgeons

LEVY LIBRARY MT. SINAI MEDICAL CENTER

Theme Issues

MAY 1992

Theme Section: Management of Malignant Melanoma
Editor: Michail Shafir
plus Grand Rounds and general articles

SEPTEMBER 1992

Human Cancer: From Precurable to Curable
Editors: Max Sung and Takao Ohnuma

NOVEMBER 1992

Community Medicine: In Honor of Kurt Deuschle
Editors: Alan Silver and David Rose

MARCH 1993

Controversies in Neurosurgery: Cervical Spine Disorders
Editors: Martin Savitz and Kalmon Post

Available now:

Toward the Conquest of Pain
Allan P. Reed, editor
volume 58, no. 3, May 1991
84 pages **$15**

Issues in Medical Ethics: Surrogacy and Drug Use
Daniel A. Moros and Rosamond Rhodes, editors
volume 58, no. 5, October 1991
96 pages **$15**

New Concepts in Asthma
Zvi M. Marom, editor
volume 58, no. 6, November 1991
81 pages **$15**

Subscriptions for 1992: $60 individuals, U.S. and Canada; $65 all libraries; $70 individuals outside U.S. and Canada.

To order subscriptions or copies of back issues ($15 each), please send check, payable to The Mount Sinai Journal of Medicine, to the Journal at Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574; counter sales at Suite 4D, 19 E. 98th Street, New York, NY (phone: 212 241-6108; FAX: 212 722-6386).

THE
OUNT SINAI JOURNAL
F MEDICINE

UME 59 NUMBER 3 MAY 1992

Modern Management of Malignant Melanoma

Michail Shafir, editor

From the Departments of Surgery and Neoplastic Diseases of the
Mount Sinai School of Medicine (CUNY)

plus

Grand Rounds in Human Immunodeficiency Virus by
H. Clifford Lane, Joseph R. Masci, Mark J. Rosen, and Lee B. Reichman

Veterans
Affairs

BETH
ISRAEL
MEDICAL
CENTER

THE MOUNT SINAI JOURNAL OF MEDICINE

The Mount Sinai Journal of Medicine is published by The Mount Sinai Medical Center of New York and has the following affiliates: Beth Israel Medical Center, New York; Bronx Veterans Affairs Medical Center, New York; and Elmhurst Hospital Center, New York.

Editor-in-Chief

Sherman Kupfer, M.D.

Editor Emeritus

Lester R. Tuchman, M.D.

Associate Editors

Harriet S. Gilbert, M.D. Julius Wolf, M.D.

Managing Editor

Claire Sotnick

Business and Production Assistant

Claudia N. Melendez

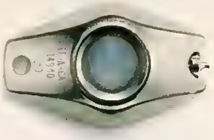
Assistant Editors

Stephen G. Baum, M.D.
David H. Bechhofer, Ph.D.
Constanin A. Bona, M.D., Ph.D.
Edward J. Bottone, Ph.D.
Jurgen Brosius, Ph.D.
Lewis Burrows, M.D.
Joseph S. Eisenman, Ph.D.
Adrienne M. Fleckman, M.D.
Richard A. Frieden, M.D.
Steven Fruchtman, M.D.
Paul L. Gilbert, M.D.
James H. Godbold, Ph.D.
Richard S. Haber, M.D.
Noam Harpaz, M.D.
Dennis P. Healy, Ph.D.
Tomas Heimann, M.D.
Barry W. Jaffin, M.D.
Andrew S. Kaplan, D.D.S.
Samuel Kenan, M.D.
Suzanne Carter Kramer, M.Sc.
Mark G. Lebwohl, M.D.
Kenneth Lieberman, M.D.
Charles Lockwood, M.D.
Steven Markowitz, M.D.
Bernard Mehl, D.P.S.
Myron Miller, M.D.
Edward Raab, M.D.
Allan Reed, M.D.
Allan E. Rubenstein, M.D.
David B. Sachar, M.D.
Henry Sacks, M.D.
Robert Safirstein, M.D.
Ira Sanders, M.D.
Martin H. Savitz, M.D.
Clyde B. Schechter, M.D.
Michael Serby, M.D.
Phyllis Shaw, Ph.D.
George Silvay, M.D.
Barry D. Stimmel, M.D.
Nelson Stone, M.D.
Max Sung, M.D.
Carl Teplitz, M.D.
Rein Tideiksaar, Ph.D.
Richard P. Wedeen, M.D.
Michael F. Wesson, M.D.

Editorial Board

Barry Freedman, M.B.A.
Richard Gorlin, M.D.
Nathan Kase, M.D.
Panayotis G. Katsoyannis, Ph.D.
Charles K. McSherry, M.D.
Jack G. Rabinowitz, M.D.
John W. Rowe, M.D.
Alan L. Schiller, M.D.
Alan L. Silver, M.D.
Alvin S. Teirstein, M.D.
Rosalyn S. Yalow, Ph.D.

The Mount Sinai Journal of Medicine (ISSN No. 0027-2507; USPS 284-860) is published 6 times a year in January, March, May, September, October, and November in one indexed volume by the Committee on Medical Education and Publications (Owner), The Mount Sinai Hospital, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Subscription price: individuals, U.S. and Canada, $60 per year; all libraries, $65; individuals outside the U.S. and Canada, $70. Single copies, $15. New subscriptions begin with the first issue of the calendar year. Send notice of change of address 60 days before the change is effective. Second-class postage paid at New York, NY and at additional mailing offices. POSTMASTER: Send address changes to The Mount Sinai Journal of Medicine, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Copyright 1992 The Mount Sinai Hospital.



(actual size)

It's More. It's Less. It's the Same.

P.A.S. PORT® FLUORO-FREE™

Implantable Peripheral Access System

More versatile...the P.A.S. PORT® FLUORO-FREE™ System can offer more of your patients the option of an implantable venous access system. It provides an attractive alternative to conventional access systems for oncology, AIDS, infectious diseases, hematology, pulmonary, and other patients.

More convenient and cost-effective... with the CATH-FINDER® Catheter Tracking System, the placement procedure can be performed in an outpatient surgical facility, procedure room, or operating room, for added convenience and reduced cost.

Less bulky...the peripheral arm-placed system is cosmetically appealing for patients.

Less traumatic...the peripheral system is designed to provide a less traumatic placement procedure.

Same applications...the system offers the same therapy applications and maintenance routines as conventional chest-placed venous access systems.

Same reliability...it provides the same dependability you've come to expect from the family of PORT-A-CATH® Implantable Access Systems.

And, it's FLUORO-FREE!
FLUORO-FREE catheter placement...the P.A.S. PORT® FLUORO-FREE System incorporates the CATH-FINDER Catheter Tracking System. This unique system is designed to track the catheter tip during the placement procedure without the use of fluoroscopy, reducing exposure to radiation for the surgical team and patient.

To discover more about the P.A.S. PORT® FLUORO-FREE System and the CATH-FINDER Catheter Tracking System, call us at 1-800-426-2448.

PORT-A-CATH®, P.A.S. PORT®, CATH-FINDER®, and *a better way to care*® are registered trademarks of Pharmacia Deltec Inc.
FLUORO-FREE™ is a trademark of Pharmacia Deltec Inc.
The P.A.S. PORT® FLUORO-FREE System is covered by U.S. Patent Number 4,880,414.
© 1991 Pharmacia Deltec Inc. All Rights Reserved. 8/91

Pharmacia Deltec
a better way to care
Pharmacia Deltec Inc., St. Paul, MN 55112

THE
MOUNT SINAI JOURNAL
OF MEDICINE

Volume 59
Number 3
May 1992

CONTENTS

Mount Sinai School of Medicine Awards, 1991/92 190

The Mount Sinai Journal of Medicine Awards 1991 191

MODERN MANAGEMENT OF MALIGNANT MELANOMA
Introduction *Michail Shafir* 192

Differential Diagnosis of Pigmented Lesions of the Skin
Mark Lebwohl 194

Histopathologic Features of Malignant Melanoma and Other Pigmented Lesions of the Skin *Robert G. Phelps* 198

The Practitioner's Approach to Management of an Atypical Mole *Albert M. Lefkovits* 203

Monoclonal Antibody Immunoconjugates in the Diagnosis and Treatment of Advanced Melanoma *Sushil Bhardwaj* 207

Radiological Considerations in Diagnosis of Metastatic Melanoma *Kathleen P. Halton* 211

Surgical Treatment of Melanoma: What Is Controversial and What Is Not? *Michail Shafir* 217

Chemotherapy of Metastatic Melanoma: Traditional and New Approaches *Seymour M. Cohen* 220

Role of Radiotherapy in Malignant Melanoma *Lynda Mandell* 224

Active Specific Immunotherapy with Vaccinia Melanoma Oncolysate *Marc K. Wallack, Stephen D. Scoggin, and Muthukumaran Sivanandham* 227

Humoral Antimelanoma Immunity: Induction by Mouse Antiidiotypic mAb, MK-23 and Association with Survival of Patients with Advanced Melanoma *A. Mittelman, C. Puccio, H. Chun, and T. Ahmed* 234

Immunotherapy of Metastatic Melanoma with Interleukin-2
Paolo Alberto Paciucci 238

continued

Volume 59
Number 3
May 1992

CONTENTS *continued*

GRAND ROUNDS

Immunoregulation, Immune Defects, and Clinical Strategies in HIV Infection *H. Clifford Lane* 244

Primary Care for Patients with HIV *Joseph R. Masci* 253

Pulmonary Complications of HIV Infection *Mark J. Rosen* 263

HIV Infection: The Dominant New Face of Tuberculosis *Lee B. Reichman* 271

Index to Advertisers

Pharmacia Deltec Inc. page i

Mount Sinai School of Medicine
Awards and Prizes
Academic Year 1991/92

Arthur H. Aufses, Sr., Prize in Surgery
Jane Emilie Mendez

Basic Sciences Achievement Award
Stephen Joel Slavin

Dr. Morris B. Bender Family Award in Clinical Neurology
Susan Ann Kelly

Dr. Morris B. Bender Family Award in the Neurosciences
Frederic Resnic
(Class of 1994)

Doctoral Dissertation Awards
Lorraine M. Lazar, Ph.D.
(Medical Scientist Training Program)
Richard T. Premont
(Graduate School of Biological Sciences)

Harold Elster Memorial Prize for Highest Academic Standing
Barbara Marie Owen

James Felt Memorial Prize for Highest Overall Performance
Beth Feller Printz

Dr. Alan F. Guttmacher Obstetrics & Gynecology Prize
Abbe Michelle Zaro

Saul Horowitz, Jr., Memorial Award
Steven Itzkowitz, M.D.
(Class of 1979)

Dr. Joseph R. Jagust Anesthesiology Award
Stephanie Nicole Sudikoff

George James Epidemiology Award
Sasan Azar

Dr. M. Ralph Kaufman Psychiatry Prize
Eileen Marie Kemether
Robyn Beth Rosenau

Medical Attendings Clinical Medicine Award
Rena Chase Fortier

Mount Sinai Auxiliary Board Prize for Excellence in the Study of Health Care Delivery Services
Allison Barnett Seckler

The Mount Sinai Journal of Medicine Student Prize
Matthew R. Estill

Florence L. Oppenheimer Surgery Prize
Jessica Lynne Barest

Dr. Howard R. Rappaport Pediatric Award
Julie Ann Safirstein

Arthur Ross Award for Excellence
Sharon Ruth Adler
Mathew Shane Maurer

Bela Schick Pediatric Society Prize
Wendy C. Jeshion

Solomon Silver Award in Clinical Medicine
Christine M. Eng, M.D.

The Lester R. Tuchman Awards
Michael Richard Leonard
Janice Lynn Montague

Upjohn Clinical Excellence Award
Jeffrey Harris Schneider

Excellence-in-Teaching Awards
Arthur I. Cederbaum, Ph.D.
Michael J. Klein, M.D.
Jeffrey T. Laitman, Ph.D.
Edward J. Ronan, Ph.D.

Award winners are Class of 1992 except as noted

The Mount Sinai Journal of Medicine Awards 1991

Ralph Colp Prize

The 1991 Ralph Colp Prize for best surgical paper published in *The Mount Sinai Journal* during the calendar year went to Michael J. Harrison, MD, and Narayan Sundaresan, MD, for their article, "Spinal Instrumentation for Degenerative Diseases of the Lumbar Spine" (vol. 58, no. 2). Dr. Harrison and Dr. Sundaresan are with the Department of Neurosurgery at the Mount Sinai School of Medicine (CUNY). The award was established by colleagues and friends of Ralph Colp, distinguished surgeon and for many years chief of surgery at The Mount Sinai Hospital.

Globus Prize

The 1991 Globus Prize for the best paper with a clinical orientation published in this journal during the calendar year went to Zvi M. Marom, MD, for his paper "The Role of Mast Cells in Bronchial Asthma: Mechanisms and Possible Therapeutic Indications" (vol. 58, no. 6). Dr. Marom is with the Division of Pulmonary Diseases, Department of Medicine, the Mount Sinai School of Medicine (CUNY). This award honors Joseph H. Globus, founder and first editor-in-chief (1934–1952) of *The Mount Sinai Journal.*

Student Prize

The 1991 Student Prize for the best student paper submitted to this journal during the calendar year has been awarded to Matthew R. Estill, MD, for his paper "Ileocecal Tuberculosis in an Asian Male Immigrant: Case Report and Literature Review" (in press), written when the author was a third-year student at the Mount Sinai School of Medicine (CUNY); he received his medical degree in 1992.

Introduction

Michail Shafir, MD
Associate Clinical Professor of Surgery and Neoplastic Diseases
Mount Sinai School of Medicine

Modern Management of Malignant Melanoma

MALIGNANT MELANOMA is a disease that is sui generis and has touched the deepest emotional fibers of humans throughout the years, as evidenced not only in reports by such prominent physicians as Hippocrates, René Laennec (1), and William Norris (2) but also by writers: R. L. Stevenson, for example, in *Treasure Island,* and Alexander Solshenitzyn in *Cancer Ward.* All were impressed by the growth of a "black" tumor.

This disease is increasing in frequency at an alarming rate; at present in the United States, 32,000 new cases of invasive melanoma and 6,500 deaths from melanoma are expected per year (3). It is a disease that frequently affects a young population and almost always arises only at one body site. Prompt diagnosis is crucial, because excision of an early, superficial melanoma yields a high rate of cure (4).

The etiology of melanoma remains unknown, but certain risk factors seem certain:

- fair skin that burns easily, particularly in individuals who developed intense sunburns, intermittently, at a young age (5).
- genetic: families with a high incidence of melanoma, often at a similar anatomic site in different family members, not necessarily associated with prior excessive sun exposure, have been described (6, 7). Since the recognition of increased incidence of melanoma in hosts bearing dysplastic nevi, a familial syndrome has been recognized, characterized by autosomal dominant transmission (8).

The treatment of melanoma is surgical when the disease is confined to a primary tumor. The cure rate by surgery as the single therapeutic modality decreases from close to 100% for thin melanomas to only 40% for thick lesions (6).

When the disease has disseminated, the outlook becomes grimmer, and multiple therapeutic disciplines (radiotherapy, chemotherapy, immunotherapy) are necessary. For this reason, prevention and early detection are critical. Screening programs have had encouraging results in early detection (9), along with public education emphasizing caution in sun exposure, use of sunscreen lotions, and regular examinations of the entire skin, especially in high-risk individuals (those with fair skin, light eyes, presence of multiple nevi, family history). Prompt consultation for any pigmented lesion of the skin of sudden appearance or changed character has to be encouraged. All of these measures may well be life-saving for many individuals.

References

1. Laennec R. Sur les mélanoses. Paris: Bulletin Faculté de Médecine, 1812.
2. Norris W. Eight cases of melanosis with pathological and therapeutic remarks on that disease. London: Longman, Brown, Green, Longmans and Roberts, 1857.
3. Boring CC, Squires TS, Tong T. Cancer statistics, 1991. CA 1991; 41:19.
4. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 1970; 172:902.

5. Lew RA, Sober AJ, Cook N. Sun exposure habits in patients with cutaneous melanoma: a case control study. J Dermatol Surg Oncol 1983; 9:981.
6. St. Arneault G, Nagel G, Kirkpatrick D, Holland JF. Cutaneous malignant melanoma in identical twins from a set of triplets. Cancer 1970; 25:672.
7. Cawley EP. Genetic aspects of malignant melanoma. AMA Arch Dermatol 1952; 65:440.
8. Greene MH, Tucker MA, Clark WH Jr, Kraemer KH, Elder DE, Fraser MC. High risk of malignant melanoma in melanoma-prone families with dysplastic nevi. Ann Intern Med 1985; 102:458.
9. Koh HK, Caruso A, Gage I, et al. Evaluation of melanoma/skin cancer screening in Massachusetts: preliminary results. Cancer 1990; 65:375.

Differential Diagnosis of Pigmented Lesions of the Skin

Mark Lebwohl, M.D.

Abstract

To assist in the differential diagnosis of pigmented lesions of the skin, criteria the physician can use to diagnose a variety of melanomas include size greater than 6 mm in diameter; variegation of pigmentation; asymmetry of lesions; crusting; irregular borders; and bleeding.

DIFFERENTIAL DIAGNOSIS of malignant melanoma can be difficult. However, there are guidelines which can assist the physician in this critical process; early detection can often mean the difference between life and death.

Size. Size is helpful in distinguishing a malignant melanoma from a benign nevus, the former usually arising in nevi greater than 6 mm in diameter (moles that are, approximately, bigger than the size of a pencil eraser head). Rarely, melanomas develop in nevi that are only 2–3 mm in diameter, but the vast majority are larger than that.

Shape. Shape can also signal a malignancy; any lesion that is sharply notched or indented should arouse suspicion (Fig. 1).

Pigmentation. Variegation of pigmentation is another classic feature of a malignant melanoma. While moles can be various shades of brown, a lesion that is multicolored (Fig. 2) warrants excision or, at least, biopsy. Figure 2 clearly shows a lesion with multiple colors including white, black, and reddish brown.

Symmetry. Fig. 2 also demonstrates another criterion in making a differential diagnosis: the importance of symmetry of a lesion. Moles can be bumpy (papular) or flat (macular). However, a mole that is bumpy on one side and flat on the other may indicate a malignant melanoma.

In summary, Fig. 2 reveals a lesion with all four features of malignant melanoma: variegation of pigmentation, irregular borders, asymmetry, and diameter larger than 6 mm. It was identified as a superficial spreading melanoma, which accounts for 70% of all cases of malignant melanoma.

Nodular melanoma accounts for approximately 15% of melanoma cases. Fig. 3 shows a mole that arose and rapidly developed as a nodule without significant lateral spread within the epidermis.

Crusting or Bleeding. Another sign that unfortunately appears late in the evolution of a malignant lesion is crusting or bleeding.

Acrolentiginous Melanomas. In addition to nodular and superficial spreading melanomas, those occurring on the volar aspects of the hands and feet or in the nail beds or on mucous membranes are called acrolentiginous melanomas. These carry a much more serious prognosis, even in superficial cases, and the usual associations between depth and survival do not hold true with acrolentiginous melanomas. While quite uncommon in white patients, these are relatively more common in orientals and blacks.

Adapted from the author's presentation at the seminar "Modern Management of Malignant Melanoma" held at The Mount Sinai Medical Center, March 24, 1990. From the Department of Dermatology, The Mount Sinai Medical Center, New York, N.Y.

Address correspondence and reprint requests to Mark Lebwohl, M.D., Director, Division of Clinical Dermatology, The Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029-6574.

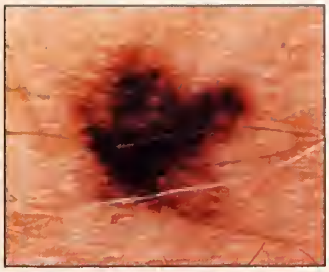


Fig. 1. Malignant melanoma: irregular border.

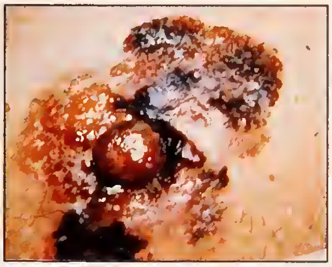


Fig. 2. Malignant melanoma: variegation of pigmentation and asymmetry.

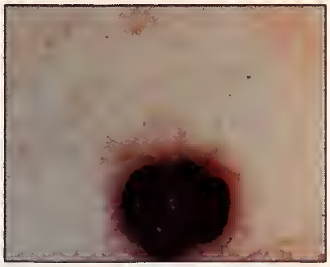


Fig. 3. Nodular melanoma: dark black color.

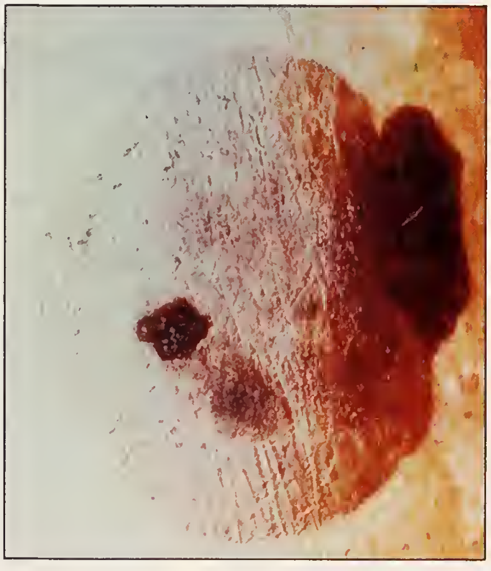


Fig. 4. Seborrheic keratosis.

Lentigo Maligna Melanoma. Lentigo maligna melanoma is a much more slowly invasive type of melanoma. It usually occurs on sun-exposed areas of the face and is classically quite large in diameter, usually several centimeters.

Difficult Diagnoses. Although the foregoing criteria are extremely useful to the physician, diagnosis can still be difficult. The lesion in Fig. 4 appeared on a lower extremity and measured 2 cm in diameter. It has a fairly irregular border, is quite notched, has a papular component in a lesion that is otherwise macular. It was biopsied and diagnosed as a seborrheic keratosis. In contrast, Fig. 5 shows seborrheic keratoses that are clearly benign and do not require biopsy.

A similar-appearing lesion from the lower abdomen (Fig. 6) measured 2.5 cm in diameter. In addition to its notched border, it had variegation of pigmentation and was diagnosed as a superficial spreading melanoma, although the patient had noticed its presence for fifteen years. Although it was located on the lower abdomen, it had features of a lentigo maligna melanoma in that it was so slow to invade and enlarge in size.

Oozing, Ulcerated Lesions. The ulcerated, oozing pedunculated growth in Fig. 7 was diagnosed as a pyogenic granuloma on the patient's thumb, a fairly characteristic location for this type of lesion. Fig. 8 shows an advanced melanoma with an oozing ulcerated nodule on the foot; pigmentation on the volar aspect of the foot revealed the malignant nature of this lesion.

Giant congenital nevi are tricky in that they can have quite irregular surfaces and develop nodules that cannot be dismissed as benign. In giant congenital nevi benign growths can be difficult to distinguish from malignant ones. The incidence of malignant degeneration in giant congenital melanocytic nevi has been estimated to range anywhere from 2% to 30%; an accurate percentage is probably slightly under 10%.

Moles on the Scalp. The scalp is an area of poor prognosis in malignant melanoma, partly because changing moles are not as readily detected at this site and are probably allowed to remain there for longer periods of time than on other parts of the body. When an atypical nevus appears on the scalp, the safest course is simply to remove it because of the possibility that it might degenerate into a melanoma.

Dysplastic Nevus Syndrome. In dysplastic nevus syndrome (Fig. 9), one can note the presence of more than one color; in Fig. 9 there is a papular component in one that is difficult to see. The mole that was biopsied turned out to be benign, but the physician must remember that the dysplastic nevus syndrome identifies patients at risk of developing malignant melanoma.

Some Benign Lesions. The lesion that appears in Fig. 10 is a nevus spilus found on the thigh of a young girl. One can note a background low level of pigmentation, which on histologic examination resembled lentigo simplex. The darker spots within it histologically resemble junctional nevi or junctional nevus cells, but this was a benign lesion.

The atypical-appearing lesion in Fig. 11 is an epidermal nevus that is benign and has no malignant potential.

Halo nevi are nevi that are surrounded by a rim of depigmentation. They are certainly benign although they can occur in patients with malignant melanomas elsewhere.

In summary, the following criteria can enable the physician to make a differential diagnosis between benign and malignant pigmented skin lesions: size greater than 6 mm in diameter; variegation of pigmentation; asymmetry of lesions; crusting; irregular borders; and bleeding.

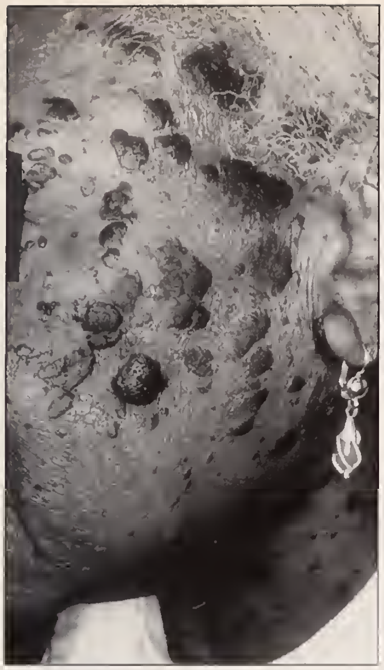
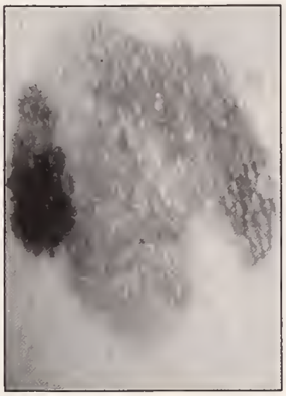


Fig. 5. Benign seborrheic keratoses.

Fig. 6. Melanoma: irregular border and variegation of pigmentation.



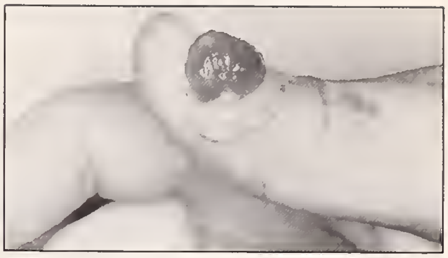


Fig. 7. Pyogenic granuloma.

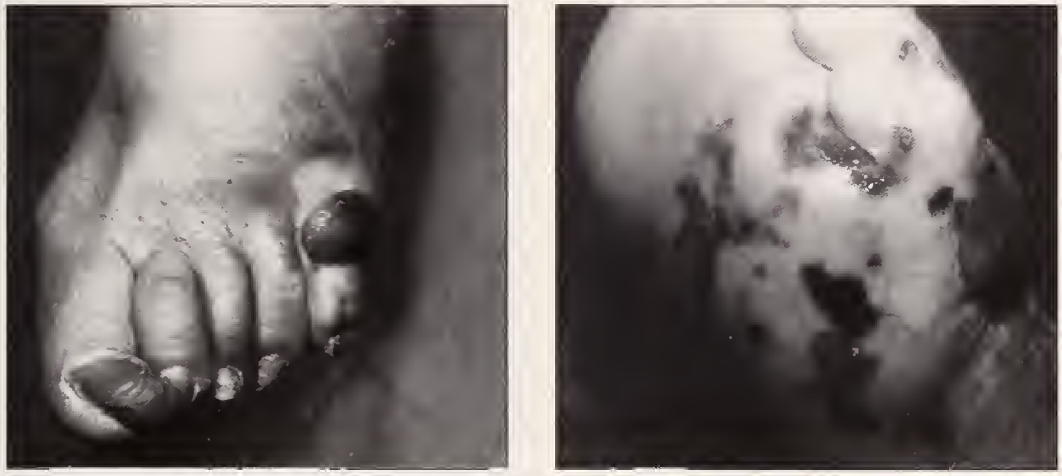


Fig. 8. Acrolentiginous melanoma: left, oozing ulcerated nodule on foot; right, malignant pigmentation on volar aspect of foot.

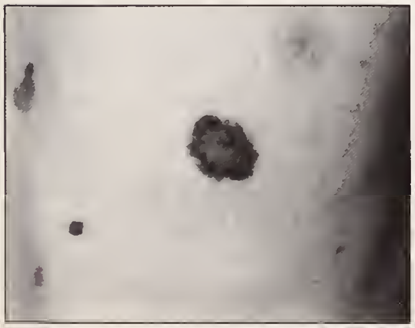


Fig. 9. Dysplastic nevus syndrome.

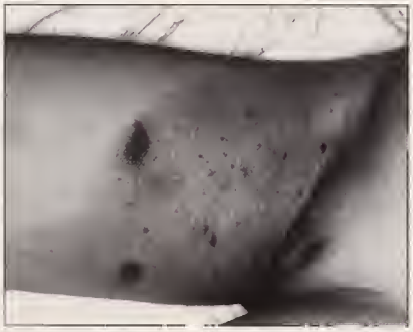


Fig. 10. Nevus spilus.

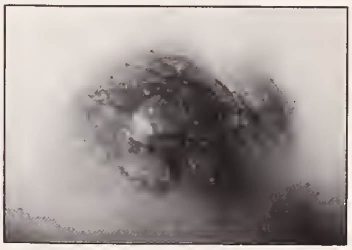


Fig. 11. Epidermal nevus.

Histopathologic Features of Malignant Melanoma and Other Pigmented Lesions of the Skin

Robert G. Phelps, M.D.

The histologic diagnosis of malignant melanoma, fortunately, in most instances is rather straightforward. This discussion primarily focuses on differential diagnosis, reviewing the histologic criteria for malignant melanoma and discussing those proliferations of melanocytes which simulate the histologic appearance of melanoma (pseudomalignancies).

Histologic Criteria for Malignant Melanoma

As elucidated by Clark, Reed, and colleagues, malignant melanoma occurs in multiple histogenetic types (1, 2). For each type, slightly different criteria must be met to make the diagnosis, but the fundamental principles remain the same. Malignant melanoma begins as a proliferation of atypical melanocytes in the epidermis. In the earliest stage an imperceptible increase in melanocytes may occur at the dermoepidermal junction. The melanocytes may have slightly enlarged nuclei. With time this hyperplasia becomes more pronounced; the melanocytes may coalesce to form nests of varying size or are present at all levels of the epidermis. This early stage or stages represents the so-called in situ phase (3).

With time, the atypical melanocytes begin to invade the dermis. This may first start as a few cells in the papillary dermis. Even at this stage, the melanoma may have no capacity for metastasis and is in what Clark and colleagues call the horizontal growth phase (4). Sometime in its evolution, malignant melanoma forms discrete nodules in the dermis; the melanoma then has entered the "vertical growth" phase and has the capacity for metastasis (5). The prognosis then depends only on the thickness of the lesion, with well-known discrete breakpoints or prognostic categories (6, 7).

Despite the similarities in the histology of malignant melanoma, four basic histogenetic types are recognized: superficial spreading, nodular, lentigo maligna, and acral lentiginous malignant melanoma. Each type has a distinctive clinical presentation and histopathology.

Superficial Spreading Type. The most common type by far is the superficial spreading variant (8). This type occurs in up to 70% of cases and occurs most frequently on the back in men and on the legs in women. Clinically, these lesions are irregularly shaped and irregularly pigmented and are often less than 2.5 cm in diameter.

Histologically, the characteristic feature is the proliferation of the so-called pagetoid melanocytes. These melanocytes have a pale cytoplasm, with a fine melanin and a central, enlarged, hyperchromatic nucleus. The melanocytes proliferate as solitary units and nests at all levels of the epidermis. With time they invade the dermis. This lesion has an in-situ or horizontal phase which is estimated to last for several years and is considered slow growing.

Nodular Type. Next most common is the nodular melanoma (8). This lesion appears as an often hyperpigmented nodule on the trunk, with very little macular component. It comprises 10%–15% of all melanomas.

Histologically, there is virtually no epider-

Adapted from the author's presentation at the seminar "Modern Management of Malignant Melanoma" held at The Mount Sinai Medical Center, March 24, 1990. From the Dermatopathology Division, Department of Surgery at The Mount Sinai Medical Center. Address correspondence and reprint requests to the author at POB 1194, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029.

mal component. Overlying and peripheral to the lesion, the epidermis may have a proliferation of atypical melanocytes not more than three rete ridges from the dermal nodule. Within the dermis, there is an expansile nodule of atypical epithelioid or spindle-shaped melanocytes. Presumably, in this lesion, the horizontal or in-situ phase was short and quickly supplemented by the vertical-growth phase.

Lentigo Maligna Type. Lentigo maligna melanoma is a less common variant of malignant melanoma which occurs in sun-exposed areas—face and hands—in elderly patients (9). Clinically it appears as a large, flat, pigmented patch of irregular shape. It often grows indolently for years without any elevation or nodules developing.

Histologically, these lesions can be subtle. To make the diagnosis, one must see at least three changes: solar elastosis, atrophy of the epidermis, and a proliferation of atypical melanocytes (10). More than any other, these lesions develop from direct ultraviolet-ray exposure. The proliferation of melanocytes in contradistinction to the other types is often histologically minimal. One may see a continuous or discontinuous proliferation of spindle-shaped or epithelioid melanocytes within the epidermis. The melanocytes often are disposed primarily in the basal epidermis and do not often exhibit the buckshot scatter of the superficial spreading type.

Acral Lentiginous Type. The acral lentiginous variant, only recently described by Reed and colleagues (2), is the least common. This variant occurs on palms, soles, and subungual sites and is least related to sun exposure. It may appear as an irregular macular lesion on the palm or sole or as a pigmented band in the nail bed. Despite its infrequency in the U.S. population, it constitutes over 70% of the melanomas in Japan and is common in both blacks and Asians.

The histology of this type is perhaps the most deceptive and can be easily missed. Within the dermoepidermal junction of a volar-plantar epidermis, one sees an increase in primarily basal melanocytes. They do not show much atypia or upward extension and proliferate primarily as solitary units. The only recognizable feature is that sometimes they have long cytoplasmic dendrites easily recognized by melanin stains (11). Despite this rather bland histology, within a short period of time these melanocytes can form an expansile dermal nodule, often of spindle cells. This dermal invasion can also be endophytic and difficult to detect clinically, the most likely explanation for the poor prognosis of such lesions.

Some melanomas cannot be easily classified using this scheme. This histogenetic classification should be viewed more as a guide than as a hard-and-fast system. Superficial spreading melanomas, for example, may occur in acral sites; and nodules ultimately occur in all types of melanoma and will replace the macular component. This intrinsic variability, however, does not necessarily mean that the classification system should be abandoned, as some authors have indicated (12).

Melanocyte Proliferations Simulating Melanoma

Conditions which simulate melanoma are in a sense pseudomalignancies. Five melanocytic proliferations are particularly known to simulate melanoma, histologically and sometimes clinically: recurrent melanocytic nevus, congenital nevus, acral nevus, dysplastic nevus, and Spitz nevus.

Recurrent Melanocytic Nevus. Melanocytic nevi are often removed or biopsied by the shave technique which may or may not be subsequently electrodesiccated. Quite often this technique fails to remove the entire nevus and therefore there is a potential for recurrence. Clinically one sees an irregular area of hyperpigmentation developing in a prior biopsy site. It may have a worrisome clinical appearance.

The histologic picture is rather characteristic (13) but can be alarming. Within the epidermis, overlying a superficial dermal scar, is a proliferation of melanocytes. They are disposed primarily in nests and show mild atypia; sometimes pagetoid extension may be evident, as well as a proliferation of solitary melanocytes. Beneath the scar are often residual dermal nevocytes. With time the epidermal melanocytes may descend into the dermis, as they would in an ordinary nevus.

Recurrences are believed to arise from residual epidermal melanocytes of the nevus, which remain in hair follicles and proliferate over the scar. The combination of this proliferation in the vicinity of a scar with persistent dermal nevocytes allows this diagnosis to be made in most instances. Given the clinical and histologic resemblance of this lesion to malignant melanoma (it is often called pseudomelanoma), the clinician and pathologist must confirm the presence of a prior lesion, and reviewing the prior biopsy slides as well is often prudent. If any uncertainty exists, the lesion should be completely and conservatively excised.

Congenital Nevi. Congenital nevi may simulate malignant melanoma histologically under two circumstances: congenital nevi that are biopsied early after birth (14, 15) and congenital nevi which form nodules during their course (16). Large congenital "bathing trunk" nevi are well known to dermatologists. They have varying definitions but usually are considered to be lesions larger than 20 cm in greatest diameter which are present at birth. They often are truncal, may have "satellite" lesions, and may have a variegated clinical appearance with differences in hue or texture.

A major concern with these lesions is that with long-term follow-up a malignant melanoma may supervene. These melanomas may be highly aggressive, may develop anywhere in the lesion, and may even arise in the dermis. Because of this concern these lesions are frequently biopsied, particularly if there is evidence of clinical change.

Histologically, if a large congenital nevus is biopsied shortly after birth, the dermoepidermal junction may show a proliferation of melanocytes. They may be pale staining and cytologically atypical and simulate the appearance of malignant melanoma in situ. The dermal portion usually shows no atypia. Familiarity with this change is essential to make the diagnosis, and misdiagnosis is easy without appropriate clinical history.

In the second pattern, an expansile dermal nodule forms in an otherwise banal population of nevocytes. The nodule can be composed of pure Schwann cells or can represent a cellular blue nevus. Sometimes it is composed of atypical epithelioid melanocytes representing an expansile clone of melanocytes. This nodule can be virtually impossible to distinguish from malignant melanoma and can only be reliably distinguished by clinical follow-up (16).

Acral Nevus. Volar and plantar nevi can appear clinically as irregular demarcated macular lesions. For unknown reasons, nevi in these locations do not mature at the same frequency as nevi in other sites and thus can still be junctional nevi even in older patients. Ordinary junction nevi have discrete nests of melanocytes at the dermoepidermal junction. They are uniform in size and shape, well demarcated, and do not show buckshot scatter or a significant proliferation of single melanocytes.

In volar and plantar nevi, the nests of melanocytes are not necessarily uniform and there is a proliferation of solitary melanocytes as well. In addition, many of the melanocytes can be found within mid epidermis (15, 17). Prominent cytologic atypia, however, is not a feature. Lesions posing some doubt should be conservatively excised.

Dysplastic Nevus. Dysplastic nevi are lesions whose concept and meaning have evolved with time. In a landmark study by Clark et al. (18), later confirmed by Lynch and colleagues (19), they were originally described in a familial setting. Essentially, patients would have multiple large atypical nevi and a tendency to develop one or several malignant melanomas. The atypical nevi were felt to be both markers of and possibly formal histogenetic precursors of malignant melanoma. Later studies of dysplastic nevi involved nonfamilial as well as familial cases (20).

In clinical appearance, dysplastic nevi have been described as often large, measuring more than 6 mm in diameter; having an ill-defined macule of erythematous border which fades into the surrounding skin; and often showing a slight admixture of colors and textures (21). In other words, they share some features with malignant melanoma, but the changes are not as severe.

Histologic reports have varied from one investigator to another (21, 22), but—culling from the literature—the following features have been considered paramount: within the dermoepidermal junction is a proliferation of melanocytes which extends laterally (aberrant differentiation); the melanocytes show focal cytologic atypia (nuclear atypia); the stroma beneath the epidermis shows a lamellated fibrosis or an infiltrate of lymphocytes (stromal changes). One feature important in its absence from this list is extreme continuous cytologic atypia or pagetoid extension, as seen in malignant melanoma. These changes occur most prominently in the lateral macular component of the nevus.

This area of dermatopathology has generated considerable controversy. Many investigators have challenged the specificity of the histologic features and the wisdom of using the term "dysplasia" for a lesion which does not often show that much atypia (23). Few deny that many or large moles may predispose to a malignant melanoma, but some investigators state that histologic melanocytic dysplasia is seen in so many nevi that its usefulness is minimal. In the most recent consensus conference, the NIH has recommended abolishing the term and replacing it with "nevus with architectural disorder," and specifying the degree of atypia (24). Only further studies will establish the significance of this.

Spitz Nevus. Spitz nevus, or nevus of large spindle-shaped or epithelioid cells, is named after Sophie Spitz, who first described it in 1948 (25). Because of the striking cytologic atypia, she originally thought the lesions were melanomas; subsequent experience has taught otherwise.

Spitz nevi usually occur in the first or second decade of life, predominantly in facial areas, but in fact can occur at any age (26, 27). Clinically they often are nonpigmented, erythematous, and look like hemangiomas. They average 5–10 mm in diameter and are well circumscribed. Pigmented variants, usually on the legs, have been described.

Histologically, Spitz nevi are among the most difficult lesions to diagnose in dermatopathology. It is said they have more features in common with malignant melanomas than they have differences. The cells comprising a Spitz nevus are spindle-shaped or epithelioid, show prominent nuclear atypia, and may proliferate in the epidermis (junctional); epidermis and dermis (compound); or dermis (intradermal). Despite the extensive atypia, the lesions can be diagnosed, utilizing specific criteria.

Spitz nevi, as opposed to malignant melanoma, are well circumscribed and symmetrical melanocytic lesions. The epidermal component is sharply circumscribed, with no lateral extension. The nests of epidermal melanocytes, despite the atypia, are usually well circumscribed and of uniform size and shape, and form discrete clefts from the surrounding epidermis. Lentiginous (single-cell) hyperplasia and pagetoid extension are not prominent.

The dermal portion is also symmetrical. The melanocytes are often nonpigmented. Telangiectasia and scattered lymphocytes may be present, but no evidence of regression or lichenoid infiltrate is present. Mitotic figures may be present, but are usually not atypical and are only superficial. The melanocytes may also mature with descent into the dermis.

A useful ancillary diagnostic feature is the presence of "Kamino" bodies (28), that is, eosinophilic globules in the epidermis or dermal papillae. These globules, believed to be necrotic melanocytes, are present in almost two-thirds of Spitz nevi and in a low percentage of malignant melanomas.

Still, even the most accomplished observers of melanocytic lesions cannot in some instances reliably distinguish some Spitz nevi from a malignant melanoma. In those cases, it is incumbent on pathologists to state their uncertainty and recommend conservative excision.

Other Simulators. Other melanocytic lesions which simulate melanoma include vulvar nevi, combined nevi, balloon cell nevi, and halo nevi. For all such lesions, the distinction from melanomas can be difficult, even to an experienced pathologist. In all such cases, conservative excision is advised, as it is better to err on the side of caution.

References

1. Clark WH Jr, From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res 1969; 29:705–726.
2. Arrington JH III, Reed RJ, Ichinose H, Krementz ET. Plantar lentiginous melanoma: a distinctive variant of human cutaneous malignant melanoma. Am J Surg Pathol 1977; 1:131–143.
3. Kamino H, Ackerman AB. Malignant melanoma in situ: the evolution of malignant melanoma within the dermis. In: Pathology of malignant melanoma, Masson's monographs in dermatopathology, vol 1. New York: Masson Publ USA, 1981; 59–91.
4. Elder DE, Guerry D IV, Epstein MN, et al. Invasive malignant melanomas lacking competence for metastasis. Am J Dermatopath 1984; 6:55–61.
5. Elder DE, Clark WH Jr. Tumor progression and prognosis in malignant melanoma. In: Elder DE, ed. Pathobiology of malignant melanoma. London: S. Karger AG 1987; 51–81.
6. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 1970; 172:902–908.
7. Day CL Jr, Lew RA, Mihm MC, et al. The nature breakpoints for primary-tumor thickness in clinical stage I melanoma (letter). N Engl J Med 1981; 305:1155.
8. Clark WH Jr, Elder DE, Van Horn M. The biologic forms of malignant melanoma. Hum Pathol 1987; 17:443–50.
9. Murphy GF, Mihm MC. Histologic reporting of malignant melanoma. In: Pathology and recognition of malignant melanoma. International Academy of Pathology monograph. Baltimore: Williams and Wilkins, 1988; 79–93.
10. Clark WH Jr, Mihm MC Jr. Lentigo maligna and lentigo-maligna melanoma. Am J Pathol 1969; 55:39–67.
11. Kerl H, Hodl S, Stettner H. Acral lentiginous melanoma. In: Pathology of malignant melanoma, Masson's monographs in dermatopathology, vol 1. New York: Masson Publ USA, 1981; 217–242.
12. Ackerman AB, David KM. A unifying concept of malignant melanoma. Hum Pathol 1986; 17:438–440.
13. Park HK, Leonard DD, Arrington JH, et al. Recurrent melanocytic nevi: clinical and histologic review of 175 cases. J Am Acad Dermatol 1987; 17:285–292.
14. Kamino H, Ackerman AB. The problem of interpreting the meanings of atypical melanocytes and unusual patterns of melanocytes within the epidermis. In: Pathology of malignant melanoma, Masson's monographs in dermatopathology, vol 1. New York: Masson Publ USA, 1981; 129–157.
15. Ackerman AB. Melanocytic proliferations that simulate malignant melanoma histopathologically. In: Pathobiology and recognition of malignant melanoma. International Academy of Pathology monograph. Baltimore: Williams and Wilkins, 1988; 153–173.
16. Kerl H. Melanocytic lesions. In: Symposium on Dermatopathology Imports, Europe and Beyond. Presented at American Society of Dermatopathology, 25th Annual Meeting, Dallas, Texas, 1991.
17. Ackerman AB et al. Differential diagnosis in dermatopathology 2. Philadelphia: Lea & Febiger, 1988; 110–113.
18. Clark WH Jr, Reimer RR, Green MH, et al. Origin of familial malignant melanomas from heritable melano-

cytic lesions: "The B-K mole syndrome." Arch Dermatol 1978; 114:732–738.

19. Lynch HT, Frichot BC III, Lynch JF. Familial atypical mole-melanoma syndrome. J Med Genet 1978; 15:352–356.
20. Elder DE, Goldman LI, Goldman SC, et al. Dysplastic nevus syndrome. Cancer 1980; 46:1787–1794.
21. Friedman RJ, Heilman ER, Rigel DS, et al. The dysplastic nevus: clinical and pathologic features. Dermatol Clin 1985; 3:239–249.
22. Elder DE, Greene MH, Bondi EE, et al. Acquired melanocytic nevi and melanoma: the dysplastic nevus syndrome. In: Pathology of malignant melanoma, Masson's monographs in dermatopathology, vol 1. New York: Masson Publ USA, 1981; 185–215.
23. Ackerman AB. What naevus is dysplastic, a syndrome and the commonest precursor of malignant melanoma? A riddle and an answer. Histopathology 1988; 13:241–256.
24. NIH Consensus Development Conference: Diagnosis and Treatment of Early Melanoma, January 27–29, 1992.
25. Spitz S. Melanomas of childhood. Am J Pathol 1948; 24:591–609.
26. Echevarria R, Ackerman LV. Spindle and epithelioid cell nevi in the adult. Cancer 1967; 20:175–189.
27. Weedon D, Little JH. Spindle and epithelioid cell nevi in children and adults: a review of 211 cases of the Spitz nevus. Cancer 1977; 40:217–225.
28. Kamino H, Misheloff E, Ackerman AB, et al. Eosinophilic globules in Spitz's nevi: new findings and a diagnostic sign. Am J Dermatopathol 1979; 1:319–324.

The Practitioner's Approach to Management of an Atypical Mole

ALBERT M. LEFKOVITS, M.D.

EACH YEAR 6,000 people in the United States die from malignant melanoma; the mean age at death has been decreasing (1), striking patients in their most productive years. In 1980, the lifetime risk of an American developing melanoma was 1 in 250. By 1990 this risk had risen to 1 in 105, and it is projected to be 1 in 75 by the year 2000—an annual increase of approximately 7%.

Risk Factors

Early Diagnosis. Malignant melanoma is the most frequent cancer of women in the United States aged 25–29 and the second most frequent cancer, after that in the breast, for women aged 30–34. However, although the incidence and absolute number of deaths from melanoma continue to rise, survival rates among patients diagnosed with melanoma have improved by 10% each decade since 1940 (2) as a result of earlier detection. The more advanced the primary tumor, the worse the prognosis. Effective early diagnosis of malignant melanoma should be able to offer complete cure of this potentially fatal cancer (3).

Consequently, the dermatologist and family practitioner should encourage every patient to be aware of the importance of undergoing a total body skin examination annually to detect malignant melanoma in its early, preinvasive stage (4).

Exposure to Sun. While every patient should be encouraged to have such a total body examination, exposure to the sun is an important risk factor, aggravated by continuing depletion of the ozone layer, which reduces the filtration of ultraviolet rays reaching the surface of the earth. Place of residence, occupational influence on exposure, and skin type all bear on the likelihood of developing malignant melanoma (5).

Fair skin (6), proximity to the equator (where sun exposure is greater), and exposure to sun at a high altitude, where atmospheric filtration of ultraviolet radiation is reduced, all increase the risk. One of the areas of the world where the population is experiencing the greatest risk of developing and dying from malignant melanoma is in Queensland, Australia (7), largely inhabited by descendants of fair-skinned Irish people living in the subtropics.

Patients with dark skin, such as blacks and people with a Mediterranean complexion, have a significantly decreased risk of developing melanoma. However, melanoma on the hands or feet of an American black is often acral lentiginous melanoma, which has a generally worse prognosis (12).

One of the great contrasts between the risk of acquiring melanoma and acquiring basal-cell or squamous-cell carcinomata is that basal- and squamous-cell carcinomata occur relatively more often in agricultural workers and sailors, who are under constant exposure to sunlight, whereas melanoma seems to occur proportionately more often in office and professional workers with rather fair-skinned complexions who vacation in Florida, Puerto Rico, and the Caribbean for brief periods, undergoing intense exposure (8). Individual severe sunburns, rather than chronic exposure to the sun, seem to be strongly correlated with the incidence of melanoma (9, 10).

Although the sun is the ultimate source of energy on the earth and can positively influence psychological mood, we must be mindful of the deleterious effects of exposure to its rays. One

---

Adapted from the author's presentation at the seminar "Modern Management of Malignant Melanoma" held at The Mount Sinai Medical Center, March 24, 1990. From the Department of Dermatology, The Mount Sinai School of Medicine. Address reprint requests to the author, Assistant Clinical Professor of Dermatology, Mount Sinai School of Medicine, Box 1047, One Gustave L. Levy Place, New York, NY 10029.

positive influence that we as physicians can have is to remind our patients to avoid sunburn; not to expose themselves to the sun between 10 AM and 3 PM, when the incidence of ultraviolet radiation is greatest; to use a sunscreen with a sun protection factor (SPF) of at least 15; and to be particularly careful to restrict unprotected sun exposure in children, since 80% of all sun exposure occurs in persons under the age of twenty.

Pregnancy. One controversial matter is the effect of pregnancy on development of subsequent melanomas in women who have previously had melanoma (11). Many oncologists feel that a woman with a history of malignant melanoma should be advised to avoid pregnancy for at least two years after giving birth.

Familial Risk. Particularly instructive in calculating a patient's risk is that melanomas can not only develop at essentially identical areas in twins, but also to an extent related to kinship in descendants, ancestors, siblings, and other relatives of patients with malignant melanoma. It has been well documented that the adult children of a middle-aged person who has had melanoma have an increased incidence of developing malignant melanoma; so also do siblings and parents of the index case. For this reason, family members of patients diagnosed as having malignant melanomas should also be encouraged to have a total body examination at periodic intervals.

Differentiating Malignant Melanomas

The average young adult has about 25 moles; even twice as many are not unusual. Moles or nevi can roughly be differentiated into intradermal nevi, which originate in the dermis of the skin; junctional nevi, originating in the epidermis; and compound nevi, with origins in both the dermis and epidermis.

Mark Lebwohl and Robert G. Phelps discuss differential diagnosis in detail; I briefly reiterate here several points.

Mnemonic for Early Detection. A good mnemonic for early detection of melanoma is ABCD:

A, asymmetry: the shape of one half does not match the other

B, borders: uneven, ragged, or notched

C, color: different shades of brown, black, or tan, or the "American flag" signs, shades of red, white, and blue

D, diameter and change in size: lesions over 6 mm must be especially suspected.

Dysplastic Nevi as Markers. First, most melanomas arise de novo, rather than from preexisting moles. However, in recent years, increasing attention has been placed on the recognition of dysplastic nevi as markers for patients at increased risk of developing malignant melanoma, and indeed, in certain cases, dysplastic nevi may indeed be precursors, especially if traumatized or sunburned. Precise definition of dysplastic nevi has been clouded by controversy, but includes a histologic pattern of disordered proliferation of melanocytes with varying degrees of atypia appearing in a lentiginous epidermal pattern, a dermal nevocellular component, and, often, failure to show maturation with descent into the dermis (13). Clinically, dysplastic nevi are distinguished from normal nevi by variegated pigmentation rather than a uniform hue, an irregular border, sometimes hazy, fading into the surrounding skin, irregular skin texture, and a diameter often over 5 mm.

Characteristically, patients with dysplastic nevi often have greater numbers of nevi than patients without such lesions. Recognition of dysplastic nevi as precursor lesions of melanoma in families was first noted in 1978 (14), and a recent review of nevomelanocytic specimens at a major institution reported a marked increase in the percentage and absolute increase in numbers of histopathologic diagnoses of dysplastic nevi in major teaching hospitals (15).

Spontaneous Regression. An important characteristic of malignant melanoma is that certain lesions show spontaneous regression, clinically manifested by areas of whitened skin within or adjacent to melanomas. This is evidence of a strong lymphohistiocytic response. Although this indicates an intact immune response to the melanoma itself, unfortunately lesions with evidence of spontaneous regression often have a worse prognosis than lesions without such changes.

Another factor to remember is that lesions on the so-called BANS area, that is to say the back, axillae, neck, and scalp, tend to have a worse prognosis than melanomas on the extremities.

Variants of Potentially Malignant Lesions. Two of the many variants of potentially malignant lesions are congenital nevi and lentigo maligna.

A pigmented nevus documented as present at the birth of the patient rather than acquired after birth has a greater chance of being a melanoma. While controversy exists over the likelihood of congenital nevi being malignant, it is generally agreed that large congenital nevi carry a high lifetime risk of becoming malignant.

Congenital nevi are found in about 1% of newborns; most of these nevi are smaller than 3 or 4 cm. Giant pigmented congenital nevi, 10 cm or larger, are present in less than 1 in 20,000 newborns (16) and have a high risk of malignant transformation.

Affecting persons at the other end of the age spectrum, lentigo maligna melanoma, also called Hutchinson's freckle, occurs primarily on sun-damaged skin of older individuals, and may be regarded as an early melanoma. Characteristically it slowly develops from a radial to a vertical growth phase, but has full potential to develop into a superficial spreading, frankly invasive, or even a nodular melanoma.

Acral lentiginous melanomas appear on the palms and soles, or under the nail plate, in which case it is termed subungual melanoma. These forms are more common in dark-skinned than in light-skinned patients (17).

The Clinical Approach

With these facts in mind, how does the clinician approach the patient with a suspected lesion?

History. First of all, as in any other type of medical examination, obtain a good history. Find out if there has been any personal or family history of melanoma, including occurrence among parents, siblings, children, and cousins. Determine whether any nevi are congenital. Ask the patient about history of sun exposure, sunburn, and ease of tanning. Inquire about trauma to nevi from shaving and fitting of clothing, especially undergarments.

Examination of Skin Surface. Second, thorough and extensive examination of the entire skin surface should be undertaken, especially behind the ears, between the toes, and around the genitals. If it is awkward to examine the genitals in a female patient, advice should be given to alert the patient's gynecologist to examine for pigmented lesions of the genitals.

Female patients have a predilection for development of melanoma over the anterior tibial areas, and both sexes should be examined with special care over the upper back.

The location and size of any dysplastic nevi should be noted on the patient's chart and should be reevaluated at the patient's next annual examination. The patient should be alerted to watch for signs of change to these nevi.

Excisional Biopsy and Margins. If a lesion is suspected of being a melanoma, the preferred course is to perform an excisional biopsy with conservative but complete margins extending into the subcutaneous fat, since this will allow determination of Clark and Breslow levels. However, on some sites, such as the face or a digit, where difficulty of primary closure or cosmetic considerations are a factor, an incisional biopsy is an acceptable alternative and does not promote dissemination of the tumor (18).

In recent years, surgical margins for thin melanomas have become more conservative (19, 20). Consequently, if the clinician is experienced in dealing with melanomas and explains the risks and alternatives to the patient, a definitive surgical procedure may be performed as the primary procedure if the lesion is relatively small and the clinician has a high degree of clinical confidence that it is a thin melanoma.

In any event, the pathologist should be asked to carefully step section the specimen and determine both Clark and Breslow levels of invasion and adequacy of lateral margins, to forecast prognosis as accurately as possible and to assess need, if any, for further studies to exclude metastatic disease and plan further therapy.

Management. Management of dysplastic nevi remains controversial. In patients with multiple dysplastic nevi, excisional biopsy of all dysplastic nevi is impractical and unnecessary. For clinically apparent severely dysplastic nevi, conservative excision with 2–3 mm margins, or a deep shave excision removing all nevus cells, is appropriate (13). As with melanoma, these specimens should also be pathologically examined by step sectioning. Patients with dysplastic nevi should be carefully followed up and should themselves be educated about watching for changes in lesions, so that if such changes occur before the time of the next scheduled appointment, the patient will be alerted to return for a prompt examination and evaluation.

Finally, patients with melanoma should be instructed to have their siblings, parents, and children examined for early screening and detection of melanomas.

With programs of thorough examination and screening and education about avoiding excessive sun exposure, we can hope to curtail the tragic loss of life from this dread disease.

References

1. Koh HK, Lew RA, Prout MN. Screening for melanoma/skin cancer theoretic and practical considerations. J Am Acad Dermatol 1989; 20:159–171.
2. A blueprint for community action. New York: The Skin Cancer Foundation, 1992;21.
3. Ackerman AB. No one need die of malignant melanoma. J Am Acad Dermatol 1985; 12:115–116.
4. Riegel DS, Friedman RJ, Kopf AW, et al. Importance of complete cutaneous examination for the detection of malignant melanoma. J Am Acad Dermatol 1986; 14:857–860.

5. Armstrong BK, English DR, Belch CM, Houghton AN, Milton GW, Sober AG, Soong S. Cutaneous melanoma, 2nd ed. Philadelphia: J. B. Lippincott, 1992:13–15.
6. Elwood JM, Gallagher RP, Hill GB, Spinelli JJ, Pearson JCG, Threlfall W. Pigmentation and skin reaction to sun as risk factors for cutaneous melanoma. Western Canada Melanoma Study. Br Med J 1984; 288:99–102.
7. Green A. Incidence and reporting of cutaneous melanoma in Queensland, Australia. J Dermatol 1982; 23:105–109.
8. Holman CDJ, Armstrong B. Skin melanoma and seasonal patterns. Am J Epidemiol 1981; 113:202.
9. Mackie RM, Aitchinson T. Severe sunburn and subsequent risk of primary cutaneous malignant melanoma in Scotland. Br J Cancer 1982; 46:955–960.
10. Lew RA, Sober AJ, Cook N, Marvell R, Fitzpatrick TB. Sun exposure habits in patients with cutaneous melanoma: case-control study. J Dermatol Surg Oncol 1983; 9:981–986.
11. Shiu MH, Schottenfeld D, Maclean B, Fortner JG. Adverse effect of pregnancy on melanoma. Cancer 1976; 37:181–187.
12. Reintgen DS, McCarty KS, Cox E, Seigher HF. Malignant melanoma in the American Black. Curr Surg 1983; 40:215–217.
13. Albert LS, Sober AJ. The dysplastic nevus as precursor and marker of increased risk for melanoma. In: Cutaneous Melanoma, 2nd ed. Philadelphia: J. B. Lippincott, 1992:60–69.
14. Reimer RR, Clark WH Jr, et al. Precursor lesions in familial melanoma: a new genetic preneoplastic syndrome. JAMA 1978; 239:744–746.
15. Barnhill RL, Kiryu H, Sober AJ, Mihm MC. Frequency of dysplastic nevi among nevomelanocytic lesions submitted for histopathologic examination. Arch Dermatol 1990; 126:463–465.
16. Kang S, Milton GW, Sober AJ, Belch, et al. Childhood melanoma. In: Cutaneous Melanoma, 2nd ed. Philadelphia: J. B. Lippincott, 1992: 313.
17. Reintgen DS, McCarty KM Jr, Cox E, Seigler HF. Malignant melanoma in black American and white American populations: a comparative review. JAMA 1982; 248:1856.
18. Lederman SJ, Sober AJ. Does biopsy type influence survival in clinical stage I cutaneous melanoma? J Am Acad Dermatol 1985; 13:983.
19. Ackerman AB, Soheiner AM. How wide and how deep is wide and deep enough? Hum Pathol 1983; 14:743.
20. Day CLJ, Mihm MC Jr, Sober AJ, Fitzpatrick TB, Malt RA. Narrower margins for clinical stage I malignant melanoma. N Engl J Med 1982; 306:479.

Monoclonal Antibody Immunoconjugates in the Diagnosis and Treatment of Advanced Melanoma

Sushil Bhardwaj, M.D.

Initially, I summarize here the immunolymphoscintigraphy study carried out at Mount Sinai School of Medicine, including histopathologic evidence demonstrating that the radiolabeled monoclonal antibody did indeed travel to the regional lymph nodes. I then describe experience at this institution using a ricin-A chain immunotoxin in the treatment of metastatic melanoma and discuss issues related to the repeated use of such an immunotoxin in patients.

The first patient studied in the immunolymphoscintigraphy study was a 43-year-old housewife who had 3 prior neoplasms, including myeloblastoma, mesothelioma of the pelvis, for which she had received pelvic radiation, and renal cell carcinoma. The patient had a Clark's level IV melanoma resected approximately a year before a mass in her left inguinal region was found on biopsy to be metastatic melanoma. The patient was injected with the radiolabeled monoclonal antibody ZME 018 in the webbed space of her feet bilaterally. Uptake on the ipsilateral side, and in addition significant uptake on the contralateral side, was seen. This patient then had a bilateral lymph node dissection, and did indeed have malignant melanoma on the ipsilateral side (Fig. 2) which on immunohistochemical staining did show the presence of the high-molecular-weight antigen in the lymph node. Interestingly, on the contralateral side, the area of intense uptake revealed a small focus of melanoma in a lymph node (Fig. 3). There was some speculation as to why the patient had metastasis to a contralateral inguinal lymph node. It was felt that disruption of lymphatic drainage secondary to prior pelvic radiation may have played a role. We did demonstrate that the immunolymphoscintigraphy scan added to our understanding of the extent of disease in this first patient.

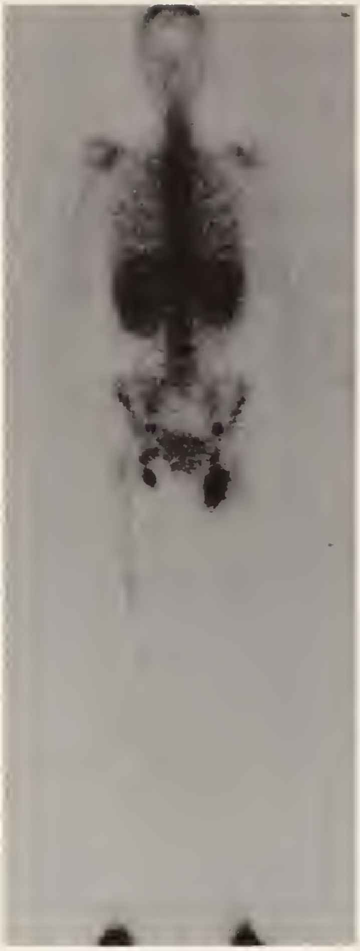
In the patients studied subsequently, there was a significant degree of nonspecific uptake. Our conclusion was that this nonspecific uptake would limit the usefulness of this technique. It was our impression that perhaps an asymmetric uptake of the monoclonal antibody when comparing the ipsilateral to the contralateral side may correlate with specific localization of the antibody in metastatic sites in the lymph nodes.

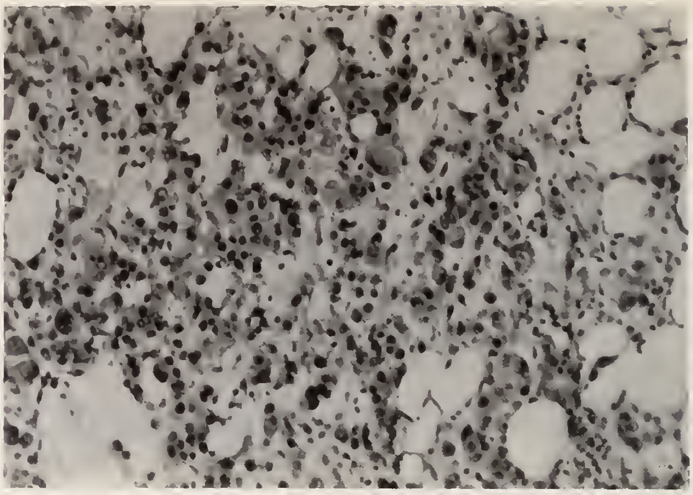
Around this time we became interested in the use of a monoclonal antibody immunotoxin in the treatment of patients with metastatic melanoma. The specific immunotoxin we worked with, Xomazyme Mel, is an immunoconjugate of a ricin-A chain molecule linked to a murine monoclonal antibody which recognizes a high-molecular-weight antigen on the surface of melanoma tumor cells. Ricin is a potent toxin derived from the castor bean. It consists of two peptide chains linked by a disulfide bond. The B chain binds nonspecifically to carbohydrate residues on the surface of cells, allowing the A chain, an extremely potent inhibitor of protein synthesis, to be internalized and to kill the cell to which the B chain has bound. If one cleaves the A chain from the B chain and then conjugates the A chain to a monoclonal antibody, it is then possible theoretically to achieve selective cell killing.

Based on this premise, initial work has been done in patients with melanoma using a ricin

---

Adapted from the author's presentation at the seminar "Modern Management of Malignant Melanoma" held at the Mount Sinai Medical Center on March 24, 1990. Address reprint request to the author at Box 1129, Department of Neoplastic Diseases, Mount Sinai Medical Center, One Gustave Levy Place, New York, New York 10029.





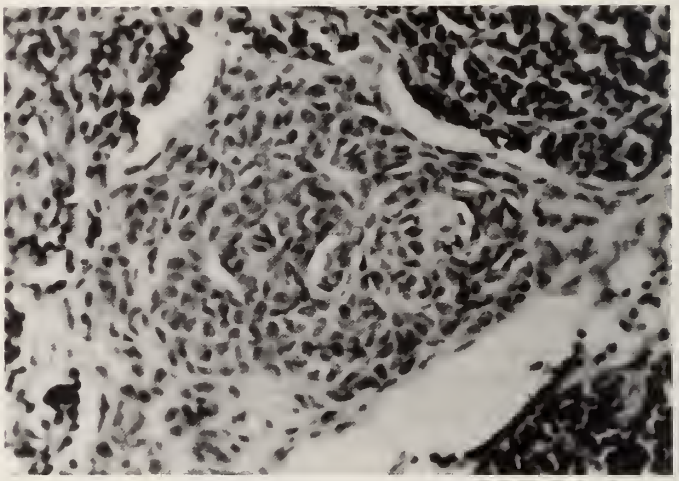


Fig. 1. Left Immunolymphoscintigraphic scan 24 h following interdigital injection of ZME 018. **Fig. 2. Right top** Focus of melanoma visualized in left inguinal area (ipsilateral side) by ZME 018 scan. **Fig. 3. Right bottom** Focus of melanoma in lymph node visualized in right inguinal area (contralateral side) by ZME 018 scan.

A-chain immunotoxin (Fig. 4). Antimelanoma immunotoxin should have these characteristics: (a) retain binding specificity of parent antibody; (b) be specifically cytolytic for human melanoma-derived continuous cell lines; (c) mediate ADCC by human PBMC's; and (d) be active in nude mouse model of human melanoma xenografts. The ricin A-chain antimelanoma immunotoxin is characterized by

- Murine IgG_{2a} covalently coupled to ricin A chain
- Specificity to "high molecular weight antigen"
- Reacts with essentially all human melanomas, and an indium 111 conjugate images melanoma in patients with metastatic disease
- No clinically significant cross reactivity with normal tissue

Following the conjugation of the ricin-A chain to the monoclonal antibody, the binding specificity of the parent antibody is retained. The antibody is cytolytic for human melanoma-derived cell lines and is active in a nude mouse model.

The initial work was done elsewhere in a phase I study in 22 patients. In that early study there was some evidence of response. One complete response and some mixed responses were noted in patients with metastatic melanoma. The major toxicity associated with the 5-day schedule used in the phase I study was a lowering of serum albumin, which was associated with weight gain. There was some manifestation of allergic symptoms in patients who had had prior exposure to the antibody in an imaging study protocol using the same antibody as is used in the immunotoxin. The complete response was seen in a patient who had a retrocardiac mass that disappeared following treatment with one 5-day course of the immunotoxin.

It was evident from the initial study that there was development of an antimurine globulin response and of an anti-ricin A immune response.

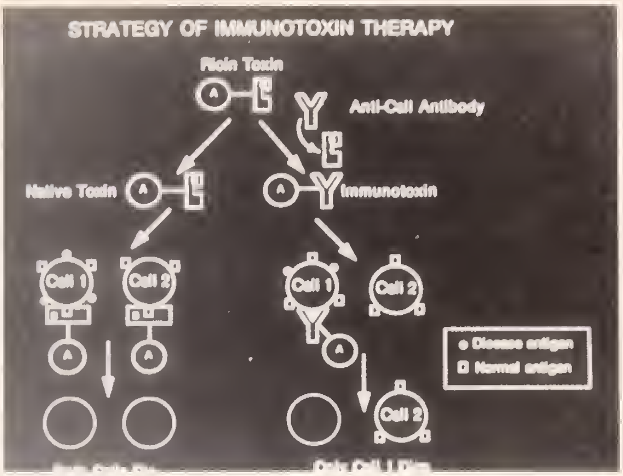

STRATEGY OF IMMUNOTOXIN THERAPY
Ricin Toxin
A
Anti-Cell Antibody
Native Toxin
A
A
Immunotoxin
Cell 1
Cell 2
A
A
Cell 1
Cell 2
A
● Disease antigen
◻ Normal antigen
Cell 2

Fig. 4. Strategy of immunotoxin therapy.

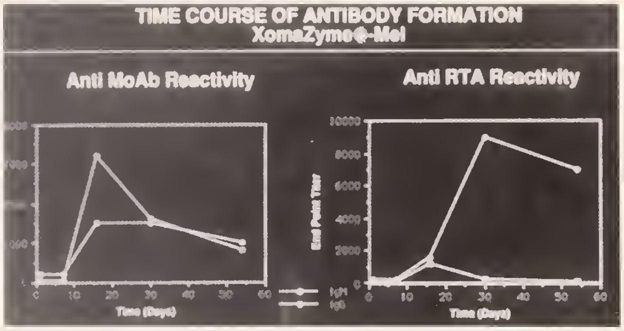
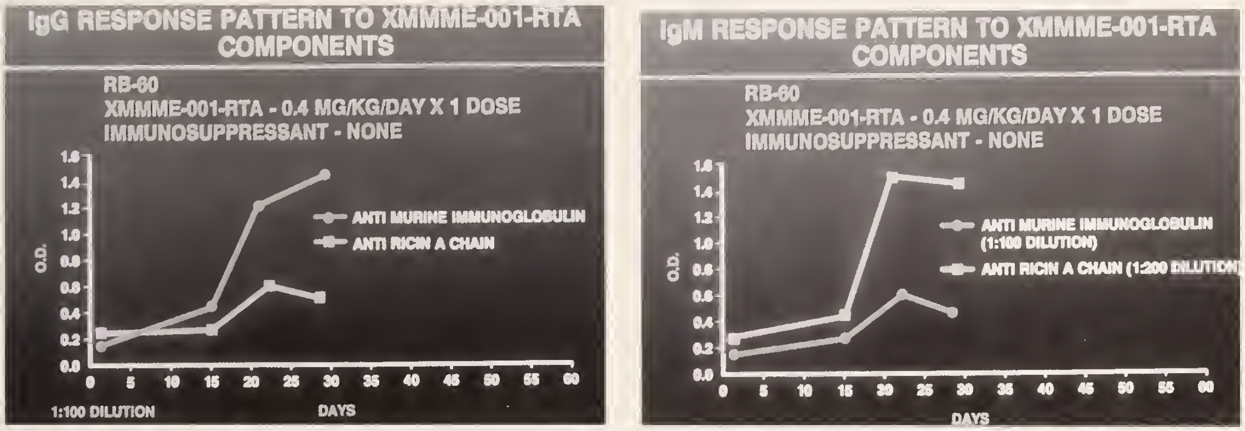
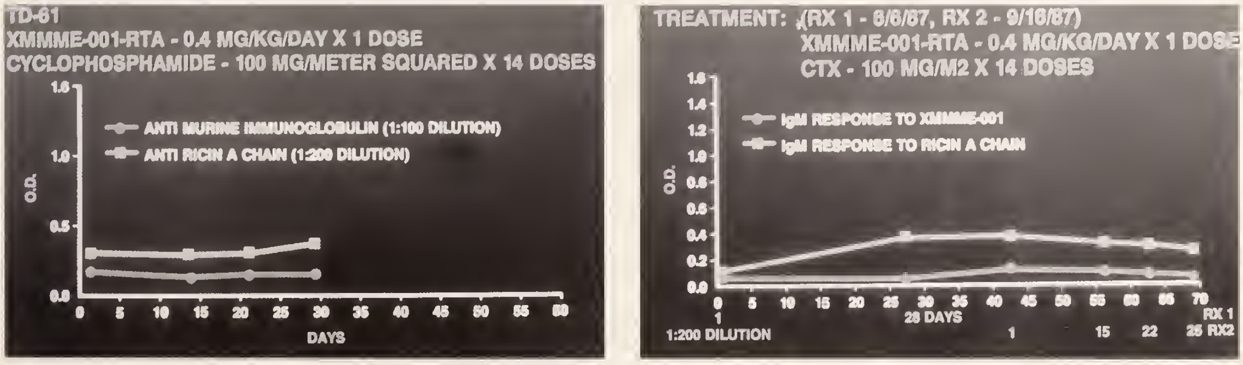

TIME COURSE OF ANTIBODY FORMATION
XomaZyme®-Mel

Fig. 5. Development of immune response to monoclonal antibody (MoAb) and ricin-A chain (RTA) components of immunotoxin.


IgG RESPONSE PATTERN TO XMMME-001-RTA COMPONENTS
RB-60
XMMME-001-RTA - 0.4 MG/KG/DAY X 1 DOSE
IMMUNOSUPPRESSANT - NONE

IgM RESPONSE PATTERN TO XMMME-001-RTA COMPONENTS
RB-60
XMMME-001-RTA - 0.4 MG/KG/DAY X 1 DOSE
IMMUNOSUPPRESSANT - NONE

Fig. 6. Development of IgG and IgM response to immunotoxin components.


TD-61
XMMME-001-RTA - 0.4 MG/KG/DAY X 1 DOSE
CYCLOPHOSPHAMIDE - 100 MG/METER SQUARED X 14 DOSES

TREATMENT: (RX 1 - 8/6/87, RX 2 - 9/16/87)
XMMME-001-RTA - 0.4 MG/KG/DAY X 1 DOSE
CTX - 100 MG/M2 X 14 DOSES

Fig. 7. Suppression of IgM response to immunotoxin components by (left) cyclophosphamide and (right) cyclophosphamide following repeated immunotoxin treatments.

This immune response was detected in a patient as early as one week following treatment with the immunotoxin (Fig. 5). It was felt that perhaps the development of these antibodies to the components of the immunotoxin would significantly interfere with the repeated use of the immunotoxin.

In an attempt to abrogate this immune response, we embarked on a study protocol using oral cyclophosphamide as an immunosuppressive at a dose of 100 mg/m^2 administered for 14 days following a single dose of immunotoxin administered intravenously in patients with metastatic melanoma. We studied a total of nine patients at The Mount Sinai Hospital. Other institutions participated in the same protocol using immunosuppressive regimens in conjunction with a similar dose of immunotoxin.

A patient who had extensive melanoma originating in the maxillary sinus and served as a control (and thus did not receive any cyclophosphamide) did have some early resolution of some of her skin nodules, but then went on to develop progressive disease within four weeks of treatment with the immunotoxin and left the protocol. This patient did mount an antibody response (Fig. 6) to the murine immunoglobulin and to the ricin-A chain component of the immunotoxin.

The second patient studied on this protocol had extensive melanoma in the gastrointestinal tract and several subcutaneous nodules. There was also demonstration of an initial therapeutic response around day 14, when some of her subcutaneous lesions had clearly regressed, and the cyclophosphamide did abrogate the antimurine immunoglobulin and anti-ricin A chain immune response (Fig. 7).

Another patient had stabilization of pulmonary metastases and received a total of 3 cycles of treatment with the immunotoxin associated with repeated administration of three 14-day cycles of oral cyclophosphamide following each treatment with the immunotoxin. It was possible to suppress the patient's immune response to the immunotoxin components by the use of the oral cyclophosphamide, which then permitted repeated administration of the immunotoxin (Fig. 7).

Another patient who received concomitant radiation therapy to a large pelvic mass did not undergo suppression of her immune response to the immunotoxin components. Based on the initial nine patients, it was our impression that cyclophosphamide may have a role in suppressing the development of an immune response to the components of the immunotoxin. It was felt that radiation therapy may diminish the effectiveness of cyclophosphamide in abrogating the immune response. In this initial trial we saw evidence of stabilization of pulmonary metastasis in one patient and partial response in pulmonary nodules in a second patient.

The cumulative experience with this antimelanoma immunotoxin in the first 205 patients studied has provided evidence of biologic activity in about 30% of patients, and evidence of objective response in about 20%. Retreatment protocols using different dosing schedules are planned.

Summary. It appears that monoclonal antibody immunoconjugates may have a role in the radioimmunodiagnosis of melanoma. The precise method of administering the immunoconjugate still remains to be defined. Also evident is the possibility of treating patients with metastatic melanoma with repeated doses of a monoclonal antibody ricin A-chain immunotoxin, but appropriate dosing schedules of the immunotoxin need to be explored further. The agents selected for suppressing the immune response need further study.

Radiological Considerations in Diagnosis of Metastatic Melanoma

Kathleen P. Halton, M.D.

The radiologic manifestations of metastatic melanoma reflect the protean and unpredictable nature of the disease itself. Melanoma can involve almost any organ system. Postmortem studies reveal its predilection for the lung, liver, central nervous system, and bone (1). There is surprisingly sparse information in the literature on radiologic studies of the patient with melanoma. In fact, there are no formal guidelines to follow in deciding which patients should be investigated for possible metastatic disease. Several authors have conducted studies to address this question.

Stage I and II Disease

Zartman and his group at the University of Colorado reviewed the imaging studies of 90 patients with newly diagnosed stage I malignant melanoma (Clark's level III and IV) (2). The imaging studies included chest x-ray, liver/spleen scan, lung scan, and either computed tomography or nuclear scan of the brain. In this series, no patients were found to have had metastatic disease at the time of initial presentation, although there were two false positive liver/spleen scans. The authors therefore concluded that only routine chest x-ray and complete blood count should be obtained in patients with newly diagnosed stage I melanoma.

In Khansur's study, 143 patients and their imaging studies were reviewed (3). The group consisted of patients with both stage I and stage II disease. All of the patients in the study had at least three of the imaging studies included in Zartman's review. In this series, once again, no patients with newly diagnosed stage I melanoma were found to have metastasis; however, nine patients with stage II disease were found to have metastatic melanoma diagnosed on chest x-ray, and nine patients with metastatic melanoma were diagnosed on liver/spleen scan.

All of the true positive abnormal chest x-rays and liver/spleen scans were in patients who had elevated levels of lactic dehydrogenase (LDH). There were three false positive chest x-rays and six false positive liver/spleen scans, the majority in patients with normal LDH levels. Once again, the authors do not recommend the routine evaluation of all patients with stage I or stage II melanoma. Instead they recommend that the investigation be determined by the patient's physical examination, symptoms, and, of course, abnormal laboratory values. The formal recommendations include routine chest x-rays, because of low radiation dose, low cost, and ready availability, and blood chemistries. In particular, they suggest the use of LDH as a possible marker for metastatic disease and suggest that patients with elevated LDH levels should have a thorough investigation for metastasis even if asymptomatic.

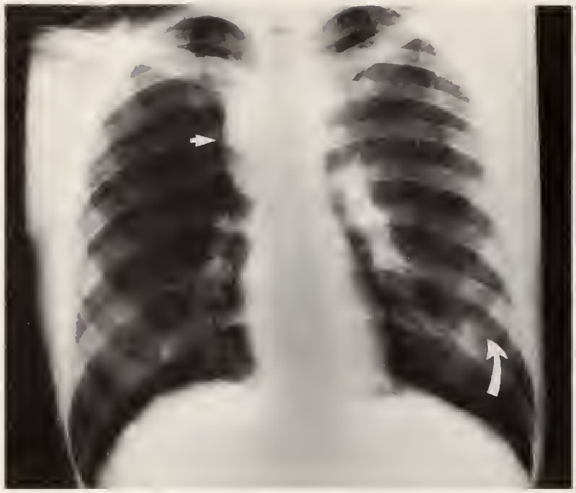
To summarize, patients with Stage I and Stage II melanoma do not necessarily require thorough investigation with radiologic imaging unless physical examination findings or laboratory values are abnormal. Most authors do recommend routine chest x-rays.

Stage III and IV Disease

Patients with stage III disease, that is, those with high risk for metastasis and, of course, patients with stage IV disease generally require multiorgan imaging to identify metastasis, deter-

---

From the Department of Radiology, The Mount Sinai Medical Center (CUNY), New York. Address reprint requests to the author at Box 1234, Department of Radiology, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029-6574.



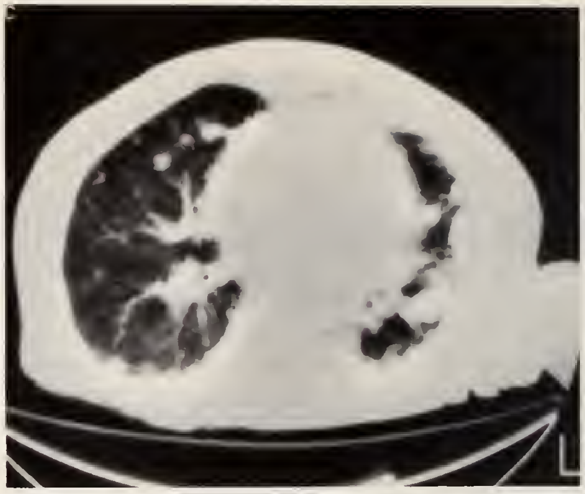
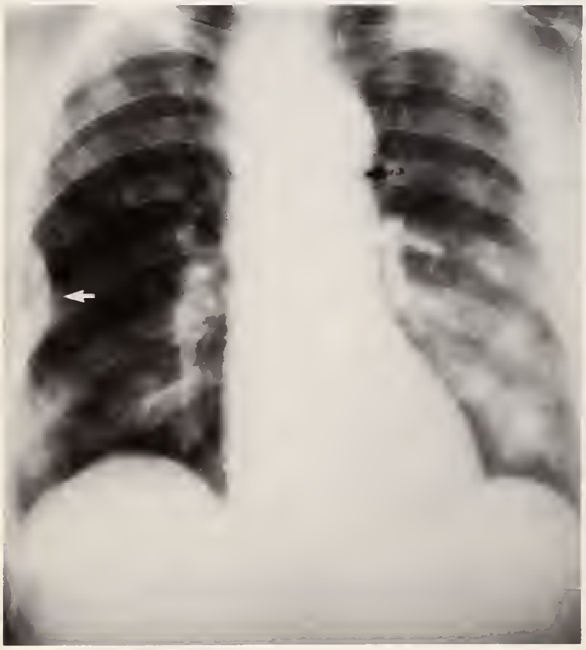


Fig. 1. **Left** Wide mediastinum secondary to adenopathy (arrow) and left lung nodule (curved arrow) in a patient with metastatic melanoma. Fig. 2. **Right** Computed tomography in a different patient reveals multiple lung nodules.

mine the extent of disease, and provide a baseline in assessing response to future treatment. Recent technological advances have given us a multitude of imaging modalities by which to evaluate patients for possible metastatic disease. The choice of imaging modality depends more on the organ system being imaged than on the particular neoplasm. Chest x-ray can identify both parenchymal nodules and mediastinal adenopathy (Fig. 1); however, a study from Duke University (4) demonstrates the superior sensitivity and overall accuracy of CT scanning of the thorax as compared to both plain chest x-ray and tomography (Fig. 2).

Chest x-ray can also be helpful in the evaluation of the bony thorax. On one chest x-ray, for example, a mass is noted which may involve a rib; a second film performed with a rib technique demonstrates the expansile lytic lesion does involve a lower right rib, and it was confirmed to be metastatic melanoma (Figs. 3, 4).

Osseous metastasis from melanoma can involve any part of the skeleton. For example, one



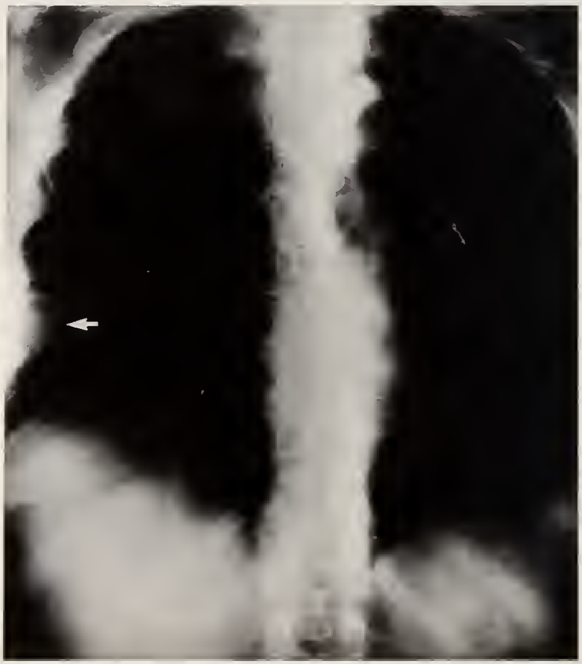
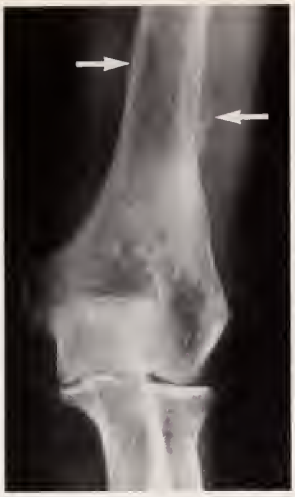


Fig. 3. **Left** Chest x-ray reveals pleural-based mass at right lung base (arrow) that may involve rib. Fig. 4. **Right** Repeat x-ray performed with bone technique reveals mass to be expansile lytic lesion in a lower right rib.



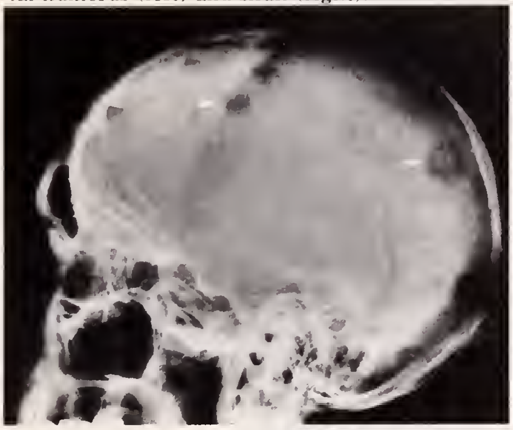


Fig. 5. Skeletal survey in patient with metastatic melanoma revealed osseous involvement of both distal humerus (left) and skull (right).

patient proven to have osseous metastatic melanoma had mottled lytic changes in the distal humerus (Fig. 5) and in addition multiple lytic lesions within the skull.

Although a skull series can certainly detect bony metastasis in melanoma patients, CT scan and magnetic resonance imaging have become the primary modalities in evaluation of the head and neck. On unenhanced computed tomography of the brain, the appearance of melanoma is fairly typical. Noncontrast CT scans typically reveal a hyperdense mass. This appearance is felt to be secondary to intramural hemorrhage. Postcontrast administration reveals typically uniform enhancement of the lesion (Fig. 6). Metastatic melanoma is the third most common metastasis to involve the central nervous system and is surpassed only by lung and breast carcinoma.

Plain radiographic examination has not become completely obsolete in evaluation of the melanoma patient. For example, a routine mammography performed on a patient with a history of melanoma many years earlier revealed a fairly well circumscribed nonpalpable mass in the left breast (Fig. 7) which was proven on biopsy to be malignant melanoma.

Barium studies are still useful in evaluating the upper and lower gastrointestinal tract. In the classic, although not pathognomonic, appearance of metastatic melanoma involving the stomach, multiple lesions are seen within the stomach wall, some with central ulceration (Fig. 8). The lesions were secondary to metastatic melanoma.

On the barium enema examination in Fig. 9, a filling defect is noted within the ascending colon. The defect is secondary to an intussusception of the small intestine into the large bowel. The lead point was found to be a metastatic melanoma lesion of the small intestine, also identified in the same patient on CT scan examination (Fig. 10). However, a plain barium examination was not able to identify the heterogeneous 3-cm

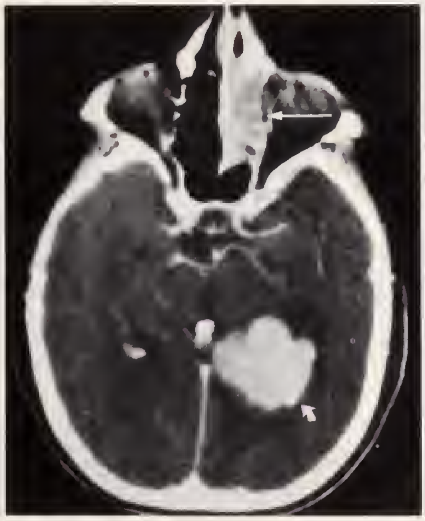


Fig. 6. Axial contrast-enhanced CT scan shows enhancing mass in left ethmoid sinuses and nasal cavity (arrow) and a second mass in occipital horn (curved arrow). Metastatic melanoma. *Courtesy of* Dr. Peter Som, NYC.

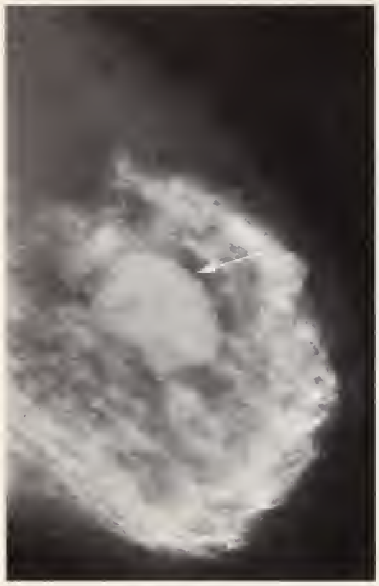


Fig. 7. Screening mammogram in woman with prior history of melanoma reveals mass in breast which proved secondary to metastatic melanoma.

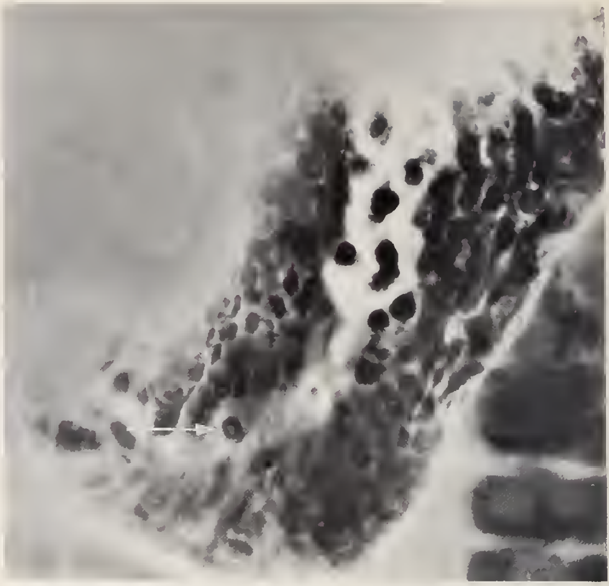


Fig. 8. Upper gastrointestinal series depicts typical appearance of metastatic melanoma involving stomach: multiple well-circumscribed intramural lesions, some demonstrating central ulceration (arrow).

mass in the right adrenal gland in this patient.

Ultrasound examination is an ideal modality for evaluation of the gallbladder and biliary tract. When metastasis involves the gallbladder, it is most frequently secondary to melanoma (Fig. 11). CT scan is not as accurate a method for evaluation of subtle lesions of the gallbladder as is ultrasound. It is, however, an excellent modality for evaluation of the liver (Fig. 12).

Ultrasound examination can certainly identify lesions within the adrenal gland. However, such an examination is time consuming (Fig. 13). CT scan in a different patient with metastatic melanoma reveals bilateral adrenal metastasis and simultaneously demonstrates multiple enhancing masses in paraspinal muscles (Fig. 14).

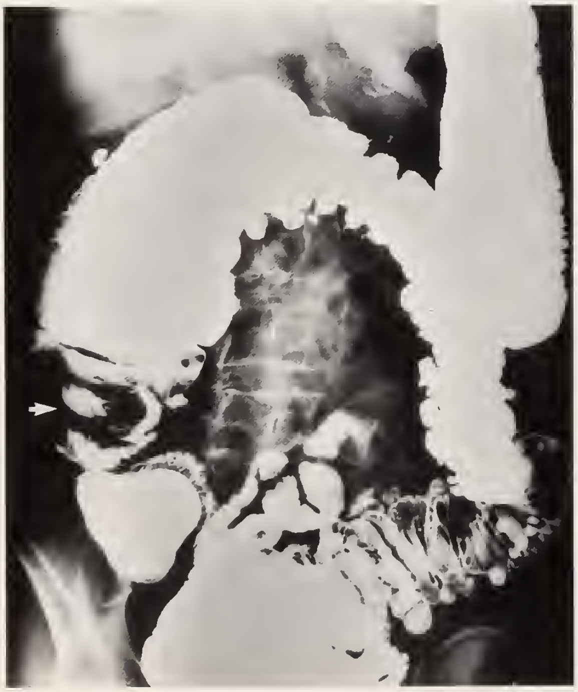
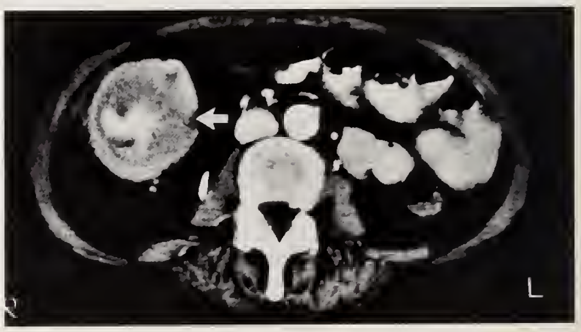
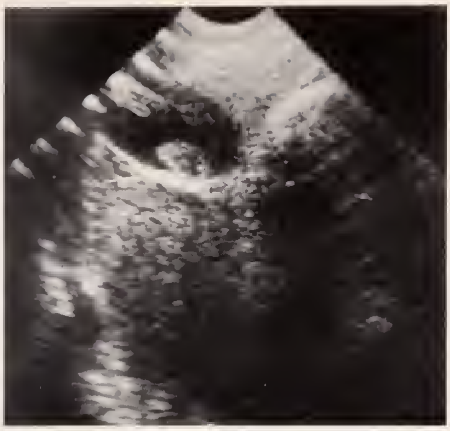


Fig. 9. **Left** Barium enema examination reveals typical "coil-spring" appearance of intussusception involving ascending colon (arrow).

Fig. 10. **Right** Computed tomography in same patient demonstrates mass in ascending colon (arrow). *Both figures courtesy of* Dr. Burton Cohen, NYC.





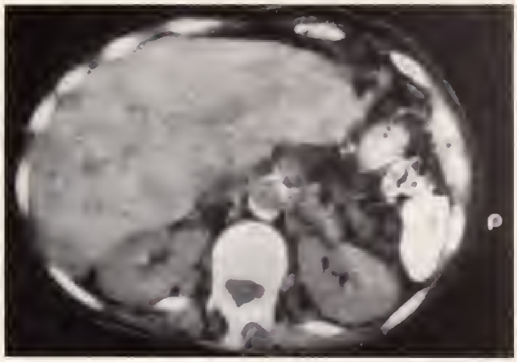
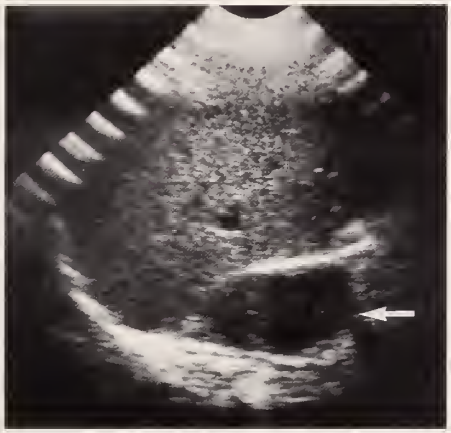


Fig. 11. **Left** Ultrasonography reveals unusual finding, mass (arrow) arising from wall of gallbladder that proved secondary to metastatic melanoma. Fig. 12. **Right** Computed tomography in patient with metastatic melanoma reveals diffuse heterogeneity of liver secondary to metastatic infiltration.



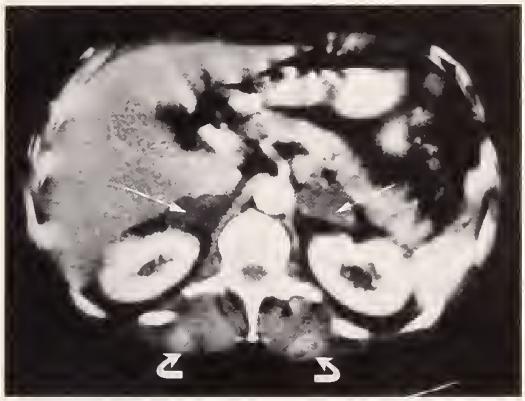
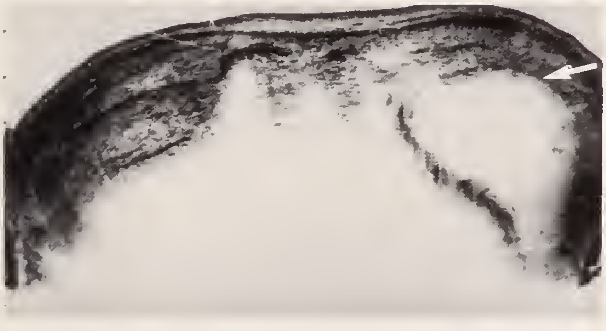


Fig. 13. **Left** Ultrasonography can identify adrenal pathology: melanoma patient with bulky mass in right adrenal gland (arrow). Fig. 14. **Right** Computed tomography in another patient reveals bilateral adrenal masses (arrows) and simultaneously identifies enhancing intramuscular masses (curved arrows).



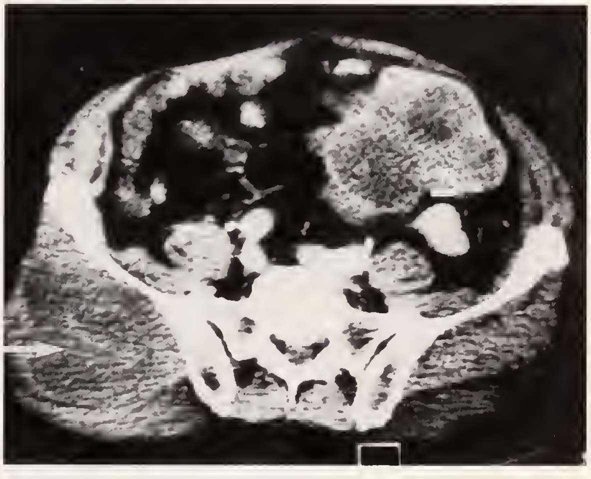


Fig. 15. **Left** Large necrotic gluteal mass identified on ultrasonography in patient scanned in prone position.
Fig. 16. **Right** Computed tomography in same patient demonstrates right gluteal mass (arrow) and bulky mesenteric lymphadenopathy (curved arrow).

Ultrasound examination in another patient demonstrates a large necrotic lesion in the gluteal muscle (Fig. 15). The same finding is easily demonstrated on CT scan examination, and at the same time large necrotic, mesenteric lymph nodes are identified in this patient with metastatic melanoma (Fig. 16).

As these cases demonstrate, CT scan provides a rapid and noninvasive means of evaluating the entire abdomen and pelvis. It is also an excellent survey examination of the thorax.

The role of MRI scanning in the abdomen and thorax is still evolving. It is sensitive in evaluation of the mediastinum but is not useful in detection of parenchymal nodules. Although it is an excellent modality for evaluation of the liver, its utility remains limited in evaluating the gastrointestinal tract. However, with the development of newer techniques designed to eliminate image degradation from problems such as cardiac, respiratory, and peristaltic motion, the modality may soon play a larger role in the detection of metastatic disease.

References

1. Akslen LA, Hove LM, Hartveit F. Metastatic distribution in malignant melanoma. Invasion Metastasis 1987; 7:253–263.
2. Zartman GM, Thomas MR, Robinson WA. Metastatic disease in patients with newly diagnosed malignant melanoma. J Surg Oncol 1987; 35:163–164.
3. Khansur T, Sanders J, Das SK. Evaluation of staging workup in malignant melanoma. Arch Surg 1989; 124:847–849.
4. Heaston DK, Rodan BA, Putman CE, et al. Solitary pulmonary metastasis in malignant melanoma: a prospective comparison of conventional and computed tomography. AJR 1983; 141:169–174.

Surgical Treatment of Melanoma: What Is Controversial and What Is Not?

Michail Shafir, M.D.

Biopsy of a Pigmented Lesion Suggestive of Melanoma. The primary mainstream treatment of primary melanoma is surgical excision. Any pigmented lesion of the skin or mucosae suspected of being malignant melanoma requires excisional biopsy to establish the diagnosis and, if melanoma is confirmed, to determine the histopathologic criteria important for prognosis and treatment, those criteria being thickness, depth of invasion, histologic type, presence of ulceration, differentiation, lymphocytic infiltration and presence of regression, mitotic aggressiveness, lymphatic and vascular invasion.

The "ideal" biopsy is total excision under local anesthesia, with a narrow margin of normal-appearing epithelium and with full thickness of skin or mucosae down to subcutaneous or submucosal fat. The specimen has to be oriented, marked, and handled gently, avoiding crushing, to facilitate the pathologist's evaluation. Infiltration of the local anesthetic has to be careful and gentle, administered in a radial fashion at a distance from the obvious margins of the lesion, to avoid mechanical dispersion of melanoma cells and their possible consequent implantation or dissemination. The incision has to be oriented in the axis of a future wider excision if the diagnosis is confirmed.

In the case of round, smaller, pigmented lesions, a punch biopsy is acceptable provided the instrument, which is circular, encompasses the entire lesion.

Shave biopsies are unacceptable because they fail to encompass the full thickness of the lesion.

Incisional biopsy is an acceptable technique only under certain circumstances: if the suspect pigmented lesion is too large and cannot be excised under local anesthesia easily or in a fashion that is cosmetically satisfactory.

Wedge full-thickness biopsies may be performed in the thicker areas of the lesion as well as in any grossly ulcerated or color-variegated points.

Needle biopsy is not an acceptable technique except for cytologic evaluation of lymphadenopathy suggestive of disease, in which case a fine-needle aspiration can be done.

It is best not to evaluate the specimen(s) by frozen section because establishing the diagnosis that will govern treatment is not an emergency. For the pathologist to obtain all necessary information, paraffin-embedded tissue is better material (1–4).

Surgery of Primary Melanoma. Once the diagnosis has been confirmed by biopsy, and in the absence of metastases beyond regional lymph nodes, the definitive surgery for the primary site has to be performed. Based on the fact that melanomas grow radially and vertically and develop satellite nodules (5), a wide excision has to be undertaken. The excision has to be tridimensional, in the shape of a truncated cone down to the underlying fascial plane.

Three controversial questions have been debated in respect to margins of excision:

- how wide should the resection be?
- should the underlying fascia be excised?
- should the created defect be closed primarily or by flap or skin graft?

In the past, the recommendation was to excise a margin of skin with a radius of no less than

---

Adapted from the author's presentation at the seminar "Modern Management of Malignant Melanoma" held at The Mount Sinai Medical Center, March 24, 1990. From the Departments of Surgery and Neoplastic Diseases, Mount Sinai School of Medicine. Address reprint requests to the author, Associate Clinical Professor of Surgery and Neoplastic Diseases, 1021 Park Ave., New York, NY 10128.

5 cm and never to close the wound primarily. Any smaller margin was considered inadequate to assure the resection of melanotic cells that could constitute microscopic satellites around the primary tumor. However, the observation of successful surgery with much closer margins performed on melanomas of the face (where an excision of 5-cm radius is almost certain to produce substantial mutilation) opened the debate as to whether margins in all melanomas could be smaller.

The World Health Organization conducted a randomized study comparing 1 and 3 cm margins in melanomas thinner than 1 mm and demonstrated no difference in local recurrence (6). The question of how wide the excision of thicker melanomas should be remains unresolved and is the subject of a randomized trial. The general trend, at present, is to correlate width of resection with the size of the lesion: the thicker the lesion, the wider the excision. The ideal approach is to elevate flaps and close the defect primarily; if that is not possible, a skin graft is the preferred approach (7).

The best eclectic answer to the question, "How wide is wide?" may be "Wide enough."

The underlying fascia has also been the subject of many debates. Proponents of excision of the fascia have argued that lymphatics from the dermis traverse the fascia and that consequently a wide excision should encompass the fascia. Opponents have advanced the argument that the fascia is a solid, fibrous, relatively avascular plane that acts as a barrier to dissemination of melanoma. One study to clarify this problem showed no difference in local recurrence or in survival between patients in whom the fascia was preserved and patients in whom the fascia was excised (8).

Special considerations have to be given to melanomas arising in less common locations. For subungual sites, a distal phalanx amputation is usually necessary (9). For anorectal mucosa—fortunately a relatively rare location for melanoma—an abdominoperineal resection with wide perineal margins is often indicated (10).

Surgery of Regional Lymph Nodes. There is little debate that when involvement of regional lymph nodes is clinically evident, a complete regional lymphadenectomy with curative intent should be undertaken.

A long-standing controversy over the benefits of "prophylactic" lymph node dissection—dissection in the absence of palpable lymphadenopathies—continues to this date. Proponents of prophylactic lymphadenectomy advance the argument that up to 25% of patients without palpable nodes suggestive of melanoma will be found to have micrometastases (11). Retrospective series of patients comparing elective lymph-node dissections with follow-up observation demonstrated that patients undergoing lymphadenectomies had a clear advantage in survival (12, 13).

Opponents of prophylactic lymphadenectomy state that the benefits of this surgery have never been demonstrated and that two major prospective randomized trials have failed to demonstrate a difference in survival between groups of patients treated only with wide excision of primary melanoma and groups treated with wide excision plus prophylactic lymphadenectomy (14, 15).

Melanomas of the trunk represent a more difficult problem than lesions on the extremities, because truncal lesions can metastasize to either axillae or ilioinguinal regions. Attempts have been made to predict whether lymph-node bearing areas are at increased risk by injection of isotopes in the periphery of the primary lesion and scanning the entire areas in question; technetium-labelled human serum albumin is the agent of choice at present (16). This technique is, however, not universally accepted, and many centers propose only wide excision of the primary truncal melanoma in the absence of palpable lymph nodes.

Recently a new technique has been described to attempt to selectively determine if in a group of lymph nodes at risk there is any "sentinel" node with micrometastasis. Patent blue dye is injected in the periphery of the primary melanoma at the time of surgery, and the direction of flow of dye is observed and traced to the node most stained. This node is excised and submitted to pathologic examination; if it is metastatic, a complete lymphadenectomy of that group is performed. Thus, unnecessary major lymphadenectomies would be avoided.

This approach appears promising but is still a new technique that is not universally applied (17).

Surgery for Locally Advanced or Recurrent Melanoma. When melanoma recurs locally it may not be surgically reresectable. If it recurs as a single bulky lesion, resection may require major mutilation (for example, amputation) that may or may not control the disease.

Locoregional recurrence in the form of intransit or multiple satellite metastases can be treated by isolated limb hyperthermic perfusion. This technique has been used for more than thirty years with varying degrees of success.

There is still considerable controversy as to the value of this therapeutic modality compared to other methods such as local injections of BCG, radiotherapy, and cryotherapy. The reported statistical results, although not randomized, compare favorably to data on all other modalities (18). An ongoing randomized trial sponsored by the World Health Organization is currently assessing the efficacy of adjuvant isolation perfusion of the limbs in high-risk melanomas of the extremities.

Surgery of Metastatic Disease. Melanoma is unpredictable in its pattern of metastasis, and distant metastasis can occur with or without involvement of regional lymph nodes, shortly after the onset of the primary lesion or many years later. (A curious example: I have observed a patient who had a recurrence 24 years after a primary melanoma of the eye, the recurrence being multiple breast melanoma metastases.)

Virtually any organ or anatomic site can be affected; the most common, after skin and lymph nodes, are lung and liver, bone, and brain. Even more unusual sites are the gastrointestinal tract, pancreas, spleen, heart and pericardium, kidney, thyroid, adrenals.

When melanoma has metastasized widely, surgery can be contemplated for palliation of certain symptoms and to improve the quality of life. As examples, brain metastases can be resected, alleviating neurologic symptoms; bone metastasis with pathologic fracture risk on weight-bearing bones can be stabilized surgically. Metastasis to the small bowel can produce intestinal obstruction and gastrointestinal bleeding, both of which can be successfully treated by bowel resection.

In the case of isolated distant metastasis, long-lasting survival has been observed (19).

The issue of surgery for distant metastatic melanoma remains controversial because frequently the overall life expectancy is not long, and careful pragmatic judgments have to be made to balance the risks of surgery against the potential benefits. No strict rules can be established in this situation, and every case has to be judged on its own merit.

References

1. Harris MN, Gumport SL. Biopsy technique for malignant melanoma. J Dermatol Surg Oncol 1975; 1:24.
2. Ho VC, Milton GW, Sober AJ. Biopsy of melanoma. In: Balch CM, Houghton AN, Milton GW, Sober AJ, Soong SJ, eds. Cutaneous melanoma. Philadelphia: J. B. Lippincott, 1992:264.
3. Perry MD, Gore M, Seigler HF, Johnston WW. Fine needle aspiration biopsy of metastatic melanoma: a morphologic analysis of 174 cases. Acta Cytol 1986; 30:385.
4. Shafir R, Hiss J, Tsur H, Bubis JJ. Pitfalls in frozen section diagnosis of malignant melanoma. Cancer 1983; 51:1168.
5. Elder DE, Ainsworth AM, Clark WH Jr. The surgical pathology of primary cutaneous malignant melanoma. In: Clark WH Jr, Goldman LI, Mastrangelo MJ, eds. Human malignant melanoma. Philadelphia: Grune & Stratton, 1979:108.
6. Singletary SE, Balch CM, Urist MM, McCarthy WH, Cascinelli N. Surgical treatment of primary melanoma. In: Balch CM, Houghton AN, Milton GW, Sober AJ, Soong SJ, eds. Cutaneous melanoma. Philadelphia: J. B. Lippincott, 1992:269.
7. Aitken DR, Clausen K, Klein JP, James AG. The extent of primary melanoma excision: a re-evaluation—how wide is wide? Ann Surg 1983; 198:634.
8. Kenady DE, Brown BW, McBride CM. Excision of underlying fascia with a primary malignant melanoma: effect on recurrence and survival rates. Surgery 1982; 92:615.
9. Pack GT, Oropeza R. Subungueal melanoma. Surg Gynecol Obstet 1967; 124:571.
10. Wanebo HJ, Woodruff JM, Farr GH, Quan SH. Anorectal melanomas. Cancer 1981; 47:1891.
11. Gumport SL, Harris MN. Results of regional lymph node dissection for melanoma. Ann Surg 1974; 179:105.
12. Balch CM, Soong SJ, Murad TM, Ingellsal, Maddox WA. A multifactorial analysis of melanoma: prognostic factors in patients with stage I melanoma. Surgery 1979; 86:343.
13. Balch CM, Soong SJ, Milton GW, et al. A comparison of prognostic factors and surgical results in 1,786 patients with localized (stage I) melanoma treated in Alabama, USA, and New South Wales, Australia. Ann Surg 1982; 196:677.
14. Sim IH, Taylor WF, Pritchard DJ, Soule EH. Lymphadenectomy in the management of stage I malignant melanoma: a prospective randomized study. Mayo Clin Proc 1986; 61:697.
15. Veronesi U, Adamus J, Bandiera DC, et al. Inefficacy of immediate node dissection in stage I melanoma of the limbs. N Engl J Med 1977; 297:627.
16. Lamki LM, Haynie TP, Balch CM, Bhadkamkar VA, Podoloff DA, Kimee. Lymphoscintigraphy in the surgical management of patients with truncal melanoma: comparison of Tc sulfur colloid with Tc human-serum-albumin. J Nucl Med 1989; 30:844.
17. Morton DL, Wen DR, Cochran AJ. Intraoperative lymphatic mapping and selective lymphadenectomy for detection of regional metastases. In: Symposium on Recurrent and Regional Metastasis: Advances in the Biology and Clinical Management of Melanoma, Houston, Texas, November 1991, 53.
18. Krementz ET, Ryan RF, Muchmore JH, Carter RD, Sutherland CM, Reed RJ. Hyperthermic regional perfusion for melanoma of the limbs. In: Balch CM, Houghton AN, Milton GW, Sober AJ, Soong SJ, eds. Cutaneous melanoma. Philadelphia: J. B. Lippincott, 1992: 403.
19. Overett TK, Shiu MH. Surgical treatment of distant metastatic melanoma. Cancer 1985; 565:1222.

Chemotherapy of Metastatic Melanoma

Seymour M. Cohen, M.D., FACP

Malignant melanoma has responded to various chemotherapeutic agents (Table 1), including a 23% response rate to imidazole carboxamide (DTIC), which is considered the gold standard in single-agent chemotherapy for this disease. Often forgotten is that the response rate to DTIC is approximately 35% in women and only 8% in men. Nitrosoureas have an overall response rate of just about 15% and platinol about a 20% response rate. Smaller studies have also shown respectable response rate with procarbazine, hydroxyurea, and actinomycin-D (1–3).

In the 1970s, when single agents produced response rates, one proceeded quickly to use these agents in combination therapy (Table 2). The Southwest Oncology Group achieved a 31% response rate using a combination of BCNU, hydroxyurea, and DTIC (4). With my colleagues I reported a 42.5% response rate to a combination of BCNU, DTIC, and vincristine (5). Several groups could not duplicate this response rate, but they employed a decreased dosage of chemotherapy. Carmo-Pereira, a Portuguese investigator, repeated our trial with the same doses of chemotherapy and achieved a 35% response rate (6).

An interesting study was reported by Einhorn in which he used DTIC, the nitrosourea methyl CCNU, and vincristine in a higher dose and achieved a similar 39% response rate. The increased dosage did not increase the response rate and it resulted in a 10% drug-induced mortality rate (7).

Several researchers reported on the use of bleomycin, DeWasch et al. reported a 48% response rate to CCNU, vincristine, and bleomycin (8), and Siegler reported a 45% response rate to bleomycin, CCNU, vincristine, and DTIC (9). Other studies using similar regimens with bleomycin were unable to achieve more than the 20% response rates usual with single-agent chemotherapy. We added infusional bleomycin to our previously described BCNU, DTIC, vincristine regimen, and in 14 patients achieved one brief partial response (10).

Nathanson reported a 58% response rate with a regimen in which Velban, bleomycin, and platinum were employed in the same manner as they are in treating testicular cancer (11). A Velban dosage tolerable in younger patients with testicular cancer proved greatly toxic in some of the older patients; furthermore, the results were not duplicable by other groups, who noted at best a response rate of only 20% (12).

Nitrosourea and DNA Kinetics. Newberger and Young reported an interesting trial of patients with melanoma nodules administered a nitrosourea, methyl-CCNU; the researchers then biopsied the nodules on day 2, 3, 4, 6, and 8. They found that the maximum pertubation of DNA kinetics occurred at 48–72 hours after nitrosourea administration (13).

We therefore thought it might be judicious to treat patients with a nitrosourea, and then wait 48–72 hours before treating with other chemotherapeutic agents, in other words, at the time of maximum pertubation of DNA kinetics. The Nathanson regimen, which achieved a 58% response rate, had just been reported, and we deleted the Velban, which was quite toxic, and treated with CCNU on day 1, then with a bleomycin infusion for four days, and finally platinol.

Adapted from the author's presentation at the seminar "Modern Management of Malignant Melanoma" held at The Mount Sinai Medical Center on March 24, 1990. From Division of Medical Oncology, Dept. of Medicine, The Mount Sinai Medical Center. Address reprint requests to the author at 1045 5th Ave., New York, NY 10028.

TABLE 1
Single-Agent Chemotherapy of Malignant Melanoma

Agents	Response rate
Alkylating agents	14%
Antimetabolites	5%
Vinblastine	15%
VCR (Vincristine)	15%
Actinomycin D	17%
Procarbazine	16%
Hydroxyurea	22%
DTIC (Imidazole carboxamide)	23%
BCNU (Carmustine)	18%
Methyl CCNU	17%
CCNU (Lomustine)	13%
Adriamycin	0
Bleomycin	0
Cis-platinum	20%

TABLE 3
Response to CBP Chemotherapy

	No. (%)
Responders	
All patients	12/25 (48)
Females	10/15 (67)
Premenopausal	5/7 (71)
Postmenopausal	5/8 (63)
Males	2/10 (20)

	Mean (weeks)	Median (weeks)
Response duration (range 4–82)	26	10
Survival responders	16	14 (4 pts survived > one year)
Survival nonresponders	16	14

This regimen was repeated every six weeks. Our results (Table 3) included a 48% overall response rate; the rate was 67% in female patients, regardless of menopausal status, and only 20% in males. The median duration of response was only ten weeks; many of the patients did have far advanced disease. Median survival was 32 weeks, obviously better than in the nonresponders; four patients survived more than a year. These patients all had visceral disease.

Responses by area of involvement (Table 4) included a skin and nodal site disease response rate of 50%; but more interesting is that 8 of 11 female patients who had disease at these sites responded, whereas none of 6 males responded. Responses were also seen in liver and lung involvement.

A fascinating but unfortunate feature of the natural history of metastatic melanoma is CNS metastases. With the regimen described above, in 10 responding patients with no evidence of CNS involvement at the start of therapy, only 1 of 10 developed CNS metastases, and none of 4 patients surviving more than one year developed CNS metastases. In our previous BVD study, half of the responders eventually died of CNS metastases. This may be unique to a regimen including both CCNU and platinum, or it may be a chance occurrence (14).

TABLE 2
Combination Chemotherapy in the 1970s

Author	Agents	Response
Costanzi	BCNU, hydroxyurea, DTIC	31%
Cohen	BCNU, DTIC, VCR	42.5%
Pereira	BCNU, DTIC, VCR	35%
DeWasch	CCNU, VCR, bleomycin	48%
Einhorn	DTIC, methyl-CCNU, VCR	39%
Siegler	Bleomycin, CCNU, VCR, DTIC	45%
Beretta	DTIC, Act-D, BCNU	33%
Costanza	DTIC, Act-D	29%
Nathanson	Velban, bleomycin, platinum	58%
Green	CCNU, procarbazine, VCR, cytoxan	35%

Key to agents as in Table 1.

A regimen described by Delprete (15) (Table 5) achieved responses in 11 of 20 treated patients, 7 partial responses and 4 complete responses. Many of the patients had visceral disease, and response duration was 10 months. When tamoxifen was deleted from the regimen, only 2 of 20 patients responded (15). This study was repeated by investigators in Philadelphia, who also noted a better-than-50% response rate which dropped to 10% when tamoxifen was deleted (16). We thus have two different studies with over 60 patients achieving an approximate 50% response rate with a reasonable duration of response. In the McClay study (16), two of the complete responders have lived more than three years.

Since the ability to achieve this response seems to depend on the administration of tamoxifen, and tamoxifen when employed as a single agent in melanoma has less than a 5% response rate, one must inquire as to the reason for its

TABLE 4
Responses by Site of Involvement

	All patients	Male	Female
Liver	2/9	0/4	2/5
Lung	2/6	1/4	1/2
Skin & nodal	8/16	0/6	8/11

TABLE 5
DDBT Chemotherapy

	Dose	Timing	Length (weeks)
DTIC	220 mg/m^2	Day 1–3	4
DDP	25 mg/m^2	Day 1–3	4
BCNU	150 mg/m^2	Day 1	8
Tamoxifen	10–20 mg/m^2	bid	

Key to agents as in Table 1.

importance in this combination. It appears that tamoxifen works as a calcium antagonist, and platinol activity can be enhanced by calcium antagonists. This may thus be the mechanism for the enhanced activity of this regimen.

The M.D. Anderson Cancer Center has reported (17) that a combination regimen of vinblastine, DTIC, and platinol produced a 40% response rate, mostly partial responses, with a median duration of 6-9 mo. (17).

Taxol and Dibromodulcitol. Two new agents and an old one used in a different fashion have been recently demonstrated to be active in malignant melanoma. Taxol, the product of the Western yew, has a 15% response rate (18), and detrorubicin, a derivative of danuorubicin, has been reported to have a 19% response rate. However, in a small study of 8 previously untreated patients, all responded (19). Dibromodulcitol, a halogenated sugar with alkylating properties quite inactive when used intermittently, has recently been shown to have a 19% response rate in heavily pretreated patients when used daily (20).

Dose Intensification Trials. High-dose chemotherapy with autologous marrow infusion has yielded an overall response rate of 53%, but only 12% have been complete responders, and only rarely is remission sustained (21). Toxicity is formidable, but the use of hematopoietic growth factors could possibly reduce hematologic toxicity. However, the serious nonhematologic toxicities must be overcome. These include GI toxicity with melphalan, hepatoveno-occlusive disease with DTIC, and encephalomyelopathy, cardiomyonecrosis, and interstitial pneumonia with BCNU.

Dose intensification has also been attempted without marrow transplantation. Glover et al, employing the organic thiophosphate WR-2721, which protects normal tissue from the cytotoxic effects of alkylating agents, and a high-dose platinum regimen, achieved a 50% response rate when the platinum dosage was 100 mg/m^2. This rate increased to 69% when the dosage of platinum was increased to 150 mg/m^2 (22). However, in two other recently reported studies using cisplatinum in the dosage of 150 mg/m^2 over three days with DTIC, the response rates were 17% (Memorial Sloan-Kettering Institute) and 13% (Yale) (23,24).

Biological Modulators. There have been numerous attempts at combining chemotherapy with biological modulators such as interferon, interleukin, and BCG, but none of these attempts has shown increased therapeutic efficacy.

In my twenty years of experience in the treatment of malignant melanoma, I find today that even though obviously much must be accomplished, this disease is not unresponsive to chemotherapy; and fascinating work with biologic modulators is in progress.

References

1. Comis RL. DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 1976; 60:165–176.
2. Ahmann DL. Nitrosoureas in the management of disseminated malignant melanoma. Cancer Treat Rep 1976; 60:747–751.
3. Bellet RE, Mastrangelo MJ, Berd D, Lustbader E. Chemotherapy of metastatic melanoma. In: Clark WH, Jr, Goldman LI, Mastrangelo MJ, eds. Human malignant melanoma. New York: Grune and Stratton, 1978:325–354.
4. Costanzi JJ. DTIC (NSC-45388) studies in the Southwest Oncology Group. Cancer Treat Rep 1976; 60:189–192.
5. Cohen SM, Greenspan EM, Ratner LH, et al. Combination chemotherapy of malignant melanoma with imidazole carboxamide, BCNU, and vincristine. Cancer 1977; 39:41–44.
6. Carmo-Pereira J, Oliveira-Costa R, Pimentel P. Combination cytotoxic chemotherapy for metastatic cutaneous malignant melanoma with DTIC, BCNU, and vincristine. Cancer Treat Rep 1976; 60:1381–1383.
7. Einhorn LH, Furnas B. Combination chemotherapy for disseminated malignant melanoma with DTIC, vincristine, and methyl-CCNU. Cancer Treat Rep 1977; 61:881–883.
8. DeWasch G, Berheim J, Michel J, et al. Combination chemotherapy with three marginally effective agents, CCNU, vincristine, and bleomycin in the treatment of stage III melanoma. Cancer Treat Rep 1976; 60:1273–1276.
9. Siegler HF, Lucas VS, Pickett NJ, et al. A phase II study of bleomycin, oncovin, lomustine, and DTIC (BOLD) in stage IV metastatic melanoma. Proc Am Soc Clin Oncol 1979; 20:427.
10. Cohen SM, Ohnuma T, Cheung T, Holland JF. Bleomycin, carmustine, vincristine and decarbazine in patients with metastatic melanoma. Cancer Treat Rep 1983; 67:947–948.
11. Nathanson L, Kaufman SD, Carry RW. Vinblastine, bleomycin, platinum (VBP): a high response rate regimen in metastatic melanoma. Proc Am Soc Clin Oncol 1980; 21:479.
12. Luikart SD, Kennealey GT, Kirkwood JM. Randomized phase III trial of vinblastine, bleomycin, and cis-dichlorodiammine-platinum versus decarbazine in malignant melanoma. J Clin Oncol 1984; 14:164–168.
13. Newberger AE, Young RC. Cell kinetic changes in meta-

static melanoma following methyl CCNU. Proc Am Soc Clin Oncol 1980; 21:478.

14. Cohen SM, Ohnuma T, Ambinder EP, Holland JF. Lomustine, bleomycin and cisplatin in patients with metastatic malignant melanoma. Cancer Treat Rep 1986; 70:688–689.
15. Delprete SA, Maurer LH, O'Donnell J, Forcier RJ, Le Marbre P. Combination chemotherapy with cisplatin, carmustine, decarbazine and tamoxifen in metastatic melanoma. Cancer Treat Rep 1984; 68:1403–1405.
16. McClay E, Mastrangelo MJ, Bellet RE, Berd D. Combination chemo/hormonal therapy in the treatment of malignant melanoma. Cancer Treat Rep 1987; 71:465–469.
17. Legha SS, Ring S, Papadopoulas N, et al. A perspective evaluation of a triple drug regimen containing cisplatinum, vinblastine, and decarbazine (CBD) for metastatic melanoma. Cancer 1989; 64:2024–2029.
18. Einzig A, Trump DL, Sasloff J. Phase II pilot study of taxol in patients with malignant melanoma [Abstract]. Proc Am Soc Clin Oncol 1988; 7:249.
19. Chawla SP, Legha SS, Benjamin RS. Detrorubicin—an active anthracycline in untreated metastatic melanoma. J Clin Oncol 1985; 11:1529–1534.
20. Bellet RE, Catalano RB, Mastrangelo MJ, Berd D. Positive phase II trial of dibromodulcitol in patients with metastatic melanoma refractory to DTIC and a nitrosourea. Cancer Treat Rep 1978; 62:2095–2099.
21. Wolff SN, Herzig RH, Fay FW, et al. High-dose thiotepa with autologous bone marrow transplantation for metastatic malignant melanoma: results of phase I–II studies of the North American Bone Marrow Transplantation Group. J Clin Oncol 1989; 7:245–249.
22. Glover D, Glick J, Weiler C, Fox K, Grabelsky S, Guerry D. High dose cis-platinum: an active combination in the treatment of metastatic melanoma. J Clin Oncol 1987; 5:547–578.
23. Buzaid AC, Murrenja, Durivagh. High dose cisplatinum with decarbazine and tamoxifen in the treatment of metastatic melanoma. Cancer 1991; 68:1238–1241.
24. Steffens TA, Bajorinds A, Chapman PB et al. Phase II trial of high dose cisplatin and dacarbazine: lack of efficacy of high dose cisplatinum base therapy for metastatic melanoma. Cancer 1991; 68:1230–1237.

Role of Radiotherapy in Malignant Melanoma

LYNDA MANDELL, M.D., PH.D.

THE ROLE of radiotherapy in the management of malignant melanoma is currently undergoing reappraisal. An increasing number of clinical studies report results which refute the previously held notion that malignant melanoma is a radioresistant tumor (1–3).

Cutaneous Melanomas. The cutaneous melanomas include the lentigo maligna variant, which has been shown to be highly radioresponsive. With conventional fractionated radiotherapy at standard fraction doses of 180 to 200 cGy, the local control rates are similar to those obtained in basal and squamous cell carcinoma of the skin (4). With this same fractionation scheme, however, the response rate of the other forms of cutaneous malignant carcinoma is low.

In vitro survival curves of nodular melanoma cells reveal a wide shoulder, reflecting a large ability to repair sublethal radiation damage at low-dose fractions and suggesting the potential for better local control with larger than standard doses per fraction. As the shoulder increases on radiation dose response curves, the dose per fraction must also increase to reach the steep part of the curve where the dose response becomes logarithmic (5, 6).

Overgaard at the Institute of Research in Denmark attempted to address the question of fraction size in the radiotherapeutic management of malignant melanoma. In a series of 49 patients with lesions of either the primary site or metastatic lymph nodes, he found that those patients who received the higher dose per fraction enjoyed a statistically significant higher response rate than those who received the lower dose per fraction (Table) (7). This finding was supported by the work of other investigators (8–9).

In a 1986 update, Overgaard (10) not only confirmed his preliminary results, but also discovered that tumor volume was another important factor which influenced the outcome of radiotherapy. As the volume increased, the response rate decreased, which helped to explain the variance in response rates observed in studies in which the individual fraction dose remained constant.

Complete response translated into maintained local control and was a significant favorable prognosticator of survival outcome in patients having not only local but also regional disease at the time of radiotherapy. The relationship between local control and survival observed in such patients serves to emphasize the importance of achieving a complete response with radiotherapy (10).

Investigators from the Princess Margaret Hospital developed a radiotherapy fractionation regimen which popularized the use of high-dose fractions in the treatment of patients with malignant melanoma: 800 cGy are delivered in three fractions on days 0, 7, and 21, for a total dose of 2400 cGy. The results of this regimen were quite encouraging (11). Patients with either primary or lymph node metastases, gross or microscopic residual, enjoyed local control rates of 80%. The rates were slightly lower in patients receiving radiation for recurrent disease; however, these patients had larger tumor volumes, tending to confirm the findings of Overgaard.

Because of the poor local control in the larger tumors at maximally tolerated individual fractions, investigators began to seek ways to improve the radiotherapeutic effectiveness of radiation in these lesions without transgressing the

---

Adapted from the author's presentation at the seminar "Modern Management of Malignant Melanoma" held at The Mount Sinai Medical Center on March 24, 1990. From the Department of Radiation Oncology, The Mount Sinai Medical Center. Address reprint requests to the author, Associate Professor in that department, at One Gustave L. Levy Place, Box 1236, New York, NY 10029.

limits of normal tissue tolerance. One such way, currently in vogue, is the combined use of radiation and hyperthermia. Hyperthermia is the therapeutic use of elevated temperature and, combined with radiation, has been shown to increase the efficacy of a given dose of radiation (12). The results of trials using this combination in malignant melanoma appear promising (13, 14).

Metastatic Melanoma. Radiotherapy has proven to be effective in providing symptomatic relief in metastatic melanoma with minimal treatment-related morbidity. Reported response rates of palliation range from 40% to 80% and appear to be independent of the dose fractionation scheme and site (15–18). Currently the most commonly employed regimen is 3000 cGy delivered in 10 fractions of 300 cGy each in 10 days.

Ocular Melanomas. Currently two major radiotherapy techniques are employed to treat choroidal melanomas: brachytherapy and proton beam therapy.

In brachytherapy, plaques, within which radioisotopes are placed, are sewn into the sclera. Although cobalt 60 has classically been the radioisotope of choice (19, 20), its use is associated with significant radiation exposure. In an effort to improve on radiation safety with plaque therapy, several investigators have substituted iodine 125, which has a much lower average energy and minimal lead shielding requirement. Results of the iodine 125 trials have been excellent (21–23).

External beam machines currently commonly employed include the Cobalt machine and the linear accelerator. The entrance dose for the external beam machine is approximately 30%–40% of the prescribed dose. However, from the point of entry to 0.5–4.0 cm within the patient (the distance dependent on the energy of the radiation beam), there occurs a rapid buildup in dose which often exceeds the prescribed dose. In addition, the decline in dose from the point of dose prescription is gradual, delivering significant doses of radiation as the beam exits the patient. Thus, with such machinery, the anterior structures of the eye, which are highly radiosensitive, receive much more than the prescribed dose of radiation; those normal tissues posterior to the tumor also receive unnecessary radiation, albeit less than the prescribed tumor dose.

In proton beam therapy, the entrance dose of the primary beam is low and remains so until the prescribed depth within the patient is reached. At that point, a sudden burst of energy, the Bragg peak, occurs, emitting the desired dose of radiation to a localized area and subsequently declining rapidly in radiation deposition as the beam exits the patient. Thus, proton beam therapy allows for the sparing of normal tissues both anterior and posterior to the tumor being irradiated.

TABLE

Dose per fraction cGy	Clinical response			Overall response rate
	None	Partial	Complete	
<400	11/17	5/17	1/17	35%
400–700	6/20	11/20	4/20	70%
≥800	0/12	5/12	7/12	100%

See ref. 7.

Proton beam therapy has proven to be an effective modality of conservative treatment in choroidal melanomas. In one large series of 1006 patients, the enucleation rate was directly related to the size of the tumor. Only 2% of 99 patients with small tumors and 4% of 566 with intermediate-size lesions underwent subsequent enucleation, as compared to 10% of 341 patients with large tumors (24).

Conclusions. The purpose of this review has been to present a brief summary of the current status of radiotherapy in the treatment of malignant melanoma. With the evolving knowledge of radiobiologic principles and technical instrumentation, it is highly likely that radiotherapy may enjoy an even more active role in the overall management of this disease in the near future.

Acknowledgements

The author thanks John Calligy for his assistance in the preparation of this manuscript.

References

1. Doss LL, Memula N. The radioresponsiveness of melanoma. Int J Radiat Oncol Biol Phys 1982; 8:1131–1134.
2. Habermaltz HJ. Irradiation of malignant melanoma: experience in the past and present. Int J Radiat Oncol Biol Phys 1981; 7:131–133.
3. Harwood AR, Cummings BJ. Radiotherapy for malignant melanoma: a reappraisal. Cancer Treat Rev 1981; 8:271–282.
4. Harwood A. Conventional fractionated radiotherapy for 51 patients with lentigo maligna and lentigo maligna melanoma. Int J Radiat Oncol Biol Phys 1983; 9:1019–1021.
5. Bentzen SM, Overgaard J, Thames HD. Clinical radiobiology of malignant melanoma. Radiother Oncol 1989; 16:169–182.
6. Dewey DL. The radiosensitivity of melanoma cells in culture. Br J Radiol 1971; 44:816–817.
7. Overgaard J. Radiation treatment of malignant melanoma. Int J Radiat Oncol Biol Phys 1980; 6:41–44.
8. Habermalz HJ, Fischer JJ. Radiation therapy of malig-

nant melanoma: experience with high individual treatment doses. Cancer 1976; 2258–2262.

9. Hornsey S. The relationship between total dose, number of fractions and fraction size in the response of malignant melanoma in patients. Br J Radiol 1978; 51:905–909.
10. Overgaard J. The role of radiotherapy in recurrent and metastatic malignant melanoma: A clinical radiobiological study. Int J Radiat Oncol Biol Phys 1986; 12:867–872.
11. Johanson CR, Harwood AT, Cummings BJ. 0-7-21 radiotherapy in nodular melanoma. Cancer 1983; 51:226–232.
12. Archangeli G, Cividialli A, Nervi C, et al. Tumor control and therapeutic gain with different schedules of combined radiotherapy and local external hyperthermia in human cancer. Int J Radiat Oncol Biol Phys 1983; 9:1125–1134.
13. Kim JH, Hahn EW, Tokita N. Combination hyperthermia and radiation therapy for cutaneous malignant melanoma. Cancer 1978; 41:2143–2148.
14. Dewhirst MW, Sim DA, Forsyth K, et al. Local control and distant metastases in primary canine malignant melanomas treated with hyperthermia and/or radiotherapy. Int J Hypertherm 1985; 1:219–234.
15. Hilaris BS, Raben M, Calabrese AS et al. Value of radiation therapy for distant metastases from malignant melanoma. Cancer 1963; 16:765–773.
16. Rate WR, Solin LJ, Turrisi AT. Palliative radiotherapy for metastatic malignant melanoma: brain metastases, bone metastases, and spinal cord compression. Int J Radiat Oncol Biol Phys 1988; 15:859–864.
17. Ziegler JC, Cooper JS. Brain metastases from malignant melanoma: conventional vs. high-dose-per-fraction radiotherapy. Int J Radiat Oncol Biol Phys 1986; 12:1839–1842.
18. Katz HR. The results of different fractionation schemes in the palliative irradiation of metastatic melanoma. Int J Radiat Oncol Biol Phys 1981; 7:907–911.
19. Stallard HB. Radiotherapy for malignant melanoma of the choroid. Br J Ophthalmol 1966; 50:147–155.
20. Brady LW, Shields JA, Augsburger JJ, et al. Malignant intraocular tumors. Cancer 1982; 49:578–585.
21. Sealy R, LeRoux PLM, Rapley F, et al. The treatment of ophthalmic tumors with low-energy sources. Br J Ophthalmol 1976; 49:551–554.
22. Packer S, Rotman M. Radiotherapy of choroidal melanoma with iodine-125. Ophthalmology 1980; 87:582–590.
23. Garretson BR, Robertson DM, Earle JD. Choroidal melanoma treatment with iodine-125 brachytherapy. Arch Ophthalmol 1987; 105:1394–1397.
24. Munzenrider JE, Gragoudas ES, Seddon JM et al. Conservative treatment of uveal melanoma: probability of eye retention after photon treatment. Int J Radiat Oncol Biol Phys 1988; 15:553–558.

Active Specific Immunotherapy with Vaccinia Melanoma Oncolysate

Marc K. Wallack, M.D., Stephen D. Scoggin, M.D., and Muthukumaran Sivanandham, Ph.D.

Melanoma has significant immunologic features that correlate with the clinical course of the disease. Tumor-specific immune responses to melanoma antigens have been demonstrated by a myriad of in vitro immunological parameters. Moreover, melanoma patients with demonstrable immunocompetence have a better prognosis than those with abnormal or deficient immune responses. For example, the dinitrochlorobenzene (DNCB) skin test is an important in vivo measurement of immune competence that measures both the antigen recognition and the effector phases of an immune response. Studies to date have indicated that many melanoma patients with an increased response to DNCB reactivity have a significantly better prognosis than those patient with a diminished or absent response while on treatment (1). Therefore, these data strongly suggest that melanoma is an immunologically sensitive tumor and that a treatment strategy that augments the immunoresponsiveness of the melanoma patient might enhance survival of the same melanoma patients.

One of the more promising directions in specific immunotherapy involves the use of biologics in which the tumor cell surface has been modified by viral infection, the viral oncolysis method. Presumably the expression of viral antigens on the infected tumor cell's surface may serve to amplify the immune response of the host and promote recognition of poorly antigenic tumor determinants (2).

The first hints that viral invasion of a tumor might lead to the development of antitumor immunity were provided by the work of Sharpless (3) and Koprowski (4). Lindenmann extended these findings by inoculating influenza virus into the Ehrlich ascites tumor of A_2G mice. Within 2 to 48 hours oncolysis occurred. Animals showing definite intra-abdominal lysis of tumor were killed and explored. At exploration, these lysed intra-abdominal tumors were removed and used to immunize normal A_2G mice against the Ehrlich ascites tumor. All oncolysate-treated mice were able to resist challenge with 100X LD_{50} of the Ehrlich ascites tumor. All control animals immunized with uninfected mechanical lysate of Ehrlich ascites cells died (5). Therefore, viral oncolysates could be used to immunize mice against their tumors.

From Lindenmann's animal experiments, post-viral oncolytic immunity could be explained as a viral adjuvant effect induced by the interaction of virus-specific host-cell specific determinants (6). These determinants could mediate immunological cytolysis of tumor cells through either cellular and/or humoral immune responses.

These experiments led to the application of the method of viral oncolysis with live virus-augmented tumor cell vaccine for patients with cancer by several investigators.

Lindemann and Cavalli (7) at the University of Zurich administered allogenic acute myelogenous leukemic cells infected with fowl plague virus to 22 patients with acute myelogenous leukemia without any detectable clinical benefit. However, the preparation was inactivated with formaldehyde and the work was done in conjunction with cytoreductive chemotherapy.

Greene at St. Jude's Children's Hospital, Memphis, gave autologous and allogeneic os-

---

From the Department of Surgery, St. Vincent's Hospital and Medical Center, New York City. Address reprint request to Dr. M. Sivanandham, Department of Surgery, St. Vincent's Hospital and Medical Center, 153 West 11th Street, New York, NY 10011.

teosarcoma viral oncolysates for the immunization of 12 children with osteogenic sarcoma; the viral oncolysates were inactivated with formalin and the infecting viruses were recently isolated A or B strains of influenza virus. He reported that in patient with advanced disease the oncolysates induced antibody production to virus and tumor cell antigens without increasing cell mediated immunity. In patients with small tumor burdens both humoral and cellular immunity to autologous or allogeneic osteosarcoma cells were induced (8).

Sinkovics (9) used chemo-immunotherapy for treatment of metastatic soft tissue sarcoma. The chemotherapy consisted of Vincristine, Cyclophosphamide, and Dacarbazine. Immunotherapy consisted of inoculation of 5×10^8 viable units of BCG (Bacille Calmette-Guerin) together with allogeneic sarcoma cells infected with PR 8 influenza A virus, i.e. viral oncolysates. Of the 14 patients with metastatic sarcomas receiving comparable conventional modalities of treatment and immunotherapy with BCG and viral oncolysates, 6 had complete remission, 6 had partial remission, and 2 patients had progressive disease.

Cassel and Murray (10) of Emory University used autologous viral oncolysates prepared from melanoma cells infected with a mouse adapted neurotropic Newcastle disease virus for the immunization of patients with advanced malignant melanoma. Increased lymphocyte mediated cytotoxicity directed toward autologous cultures melanoma cells and stabilization of disease in some patients were observed (10).

Furthermore, Cassel and Murray (11) completed a phase II viral oncolysate trial in a group of 32 melanoma patients following therapeutic lymphadenectomy. These patients were not prospectively randomized. In each patient, the viral oncolysate was administered subcutaneously at regular intervals over 3 years. The cumulated progressions to disseminate disease at 1, 2, and 3 years respectively were 6%, 8% and 12% of the study group. These experienced losses were considerably lower than in the historical control groups described by other investigators. The results suggest that viral oncolysates are helpful adjuncts to surgery in the management of stage II malignant melanoma (11).

In 1972, Wallack at the Wistar Institute determined that the concept of repeated use of viral oncolysates to treat human cancer may be difficult because most oncolytic viruses are harmful to humans. As a result, we tested in vitro six live viral vaccines (measles, mumps, smallpox, rubella, yellow fever, rabies) against 4 human tumor lines (ovary, lung, melanoma, colon), and only the smallpox (vaccinia) vaccine virus duplicated the lytic action of the influenza virus on the Ehrlich ascites cells. Animal studies were subsequently undertaken with BALB/c mice using a syngeneic macrophage tumor designated as GMMSV12. These experiments demonstrated that mice immunized with an oncolysate prepared by infecting the tumor cells with vaccinia virus in vitro were completely resistant to subsequent challenge with the uninfected tumor cells, while all untreated controls developed tumors. Pre-immunization of the experimental and control groups with vaccina virus a week before the oncolysate treatment did not alter their response to the challenge with uninfected tumor cells. The specificity of this tumor protection was demonstrated in cross protection experiments and preliminary immunotherapy experiments were encouraging (12).

Therefore, vaccinia vaccine virus was chosen as a model for viral oncolysis on the basis of the above preliminary findings in animals and the fact that most humans were vaccinated to this virus.

Furthermore, experimental evidence has shown that vaccinia virus could be used as an adjuvant to generated cytotoxic T lymphocytes (CTLs); Zingernagel et al. (13) demonstrated that CTLs with dual specificity for vaccinia antigen and for H-2 antigen were generated after vaccinia infection. More recently, Hapel et al. (14) have presented an evidence that non-infectious vaccinia virus generated potent CTL responses in mice irradiated with ultra violet light. Furthermore, a recent evidence suggests that vaccinia virus induced antigens on the surface of infected cells are associated with H-2 molecules (15). This was observed even when the experiments were performed under conditions when only early vaccinia antigens were expressed on the surface of infected cells. These early antigens are reported to be sufficient for the generation of CTLs in vivo (16). Therefore, taken together, the experimental evidences strongly suggest that even though vaccinia virus is not a budding virus which presents viral antigens for immune responses as in other oncolysate work, vaccinia virus induced antigens on the surface of infected cells, which appear to be associated with H-2 molecules, are involved to stimulate immune responses. These findings encouraged us to pursue our studies using vaccinia vaccine virus oncolysates to treat human cancer.

When the mouse tumor experiments in our laboratory showed the vaccinia oncolysate to be a safe and potent immune mechanism stimulator, human experimentation was undertaken. In the first preliminary trial at the University of Pennsylvania between 1975 and 1977, advanced melanoma patients were treated with the vaccinia melanoma oncolysate (VMO) as long as they reacted to one or more common recall antigen skin tests. Results showed 0/29 patients had untoward responses; 15/29 patients had classic delayed hypersensitivity reactions at the VMO injection sites; and 9/29 with injection site reactions showed lack of progression of disease for a period beyond what would be considered normal survival for that stage of cancer. Of the 9 patients who showed some control of tumor growth, 3 patients were diagnosed as having either recurrent stage II or stage III melanoma (17).

Because of the interesting results in the melanoma patients in the preliminary trial, a formal melanoma trial was instituted at the Leon Berard Cancer Center in Lyon, France in October 1977 (18) using the same vaccinia melanoma oncolysate (VMO) from the original Pennsylvania trial. This preliminary study was undertaken for the treatment of previously treated recurrent stage II and stage III melanoma to determine the safety and efficacy of the VMO. For the purpose of this trial recurrent stage II disease was defined as recurrent tumor confined to the regional lymph nodes draining the site of the primary tumor and stage III disease was defined as melanoma disseminated to regional nodes other than the group draining the primary tumor or to subcutaneous tissue beyond the primary regional node drainage area. All patients in this trial had recurrences after receiving either BCG, DTIC (dacarbazine) or both in the surgical adjuvant setting or had been treated previously with either DTIC or BCG after multiple surgical procedures with no apparent effect in preventing recurrence. For this study, once a recurrence was found in either the patient's regional nodes or subcutaneous tissue, a staging metastatic workup was performed (chest x-ray, liver-spleen scan, brain scan, upper and lower gastrointestinal series). If the workup was negative, the patient underwent a complete resection of all grossly evident disease and was then placed on study. The VMO immunotherapy was instituted 4–6 weeks after successful surgery and consisted of weekly intradermal regional injections of 5×10^6 vaccinia altered polyvalent allogeneic melanoma cell membranes (1 mg/mL protein) each week for 20 weeks and then every 2 weeks until recurrence or 2 years. This was the same dose of VMO that was used in the Pennsylvania trial.

During the French trial, 4 melanoma tumor cell lines were used for the production of VMO. These lines (Mel. 2, Mel. 3, Mel. 4, and Mel. B) were established by the techniques of Leibovitz (19) from 4 melanoma patients at the Leon Berard Cancer Center, France. These lines were characterized as melanomas by the following criteria: (a) ultrastructure examination with the electron microscope (20); (b) tumor growth in nude mice; and (c) positive reactivity in the melanoma-specific formaldehyde-induced fluorescence assay (21). Furthermore, the melanoma-associated antigens on these 4 melanoma cell lines were defined by reacting these lines separately in a radioimmunoassay (RIA) with the Wistar melanoma monoclonal antibodies (WMMA) 19-19 and NU-4B. These melanoma monoclonal antibodies have been previously described (22). Moreover, when the reactivity of melanoma lysates (membrane extracts produced from freezing and thawing whole melanoma cells) and melanoma viral oncolysates (made with our usual method) were compared to the reactivity of non-lysed melanoma cells in RIA and WMMA, the results suggested that both the cell lysates and viral oncolysates exhibited reactivities which compared favorably to the reactivities observed with the whole cells. These results indicated that the melanoma associated antigens present on the tumor cell surface remained intact during the production of VMO. As a result, we could be certain that some putative melanoma antigens were being injected as part of the oncolysate.

The VMO therapy in this trial was monitored serologically with the *Staphylococcus* protein A (SpA) assay (23). For this assay, venous blood was obtained from the patients before treatment and every 3 months during treatment for use in the SpA assay. The SpA assay was performed by plating 1×10^3 melanoma cells into wells of micro test plates (Falcon). The melanoma target cells were obtained from the 4 lines used for VMO preparation and from two other melanoma cell lines available in the laboratory. After an overnight incubation at 37° C, the target cells were first exposed to the patient's sera, diluted 1/10, for one hour at room temperature and then exposed to SpA covalently bound to Type O human erythrocytes. The extent of rosette formation determines the endpoint of this test.

In this French trial, a total of 12 patients received VMO at a constant dose of 5×10^6 equivalents of live vaccinia vaccine virus-lysed melanoma cells per ml. Since this study was designed as a toxicity and feasibility trial, no other dosage levels were tested. None of the 12 patients included in this trial were anergic. Treatment with oncolysates at this dose level was found to be safe; there was no evidence of anaphylaxis, tumor growth at the VMO injection site, generalized vaccinia virus infection, or tumor enhancement after more than 366 injections. The minimal side effects of this therapy were fever to 39°C in 2 patients, which started one to two days after treatments and subsided by four days; pain and tenderness at the injection site in 5 patients, and an injection site dermatitis, which resembled a delayed cutaneous hypersensitivity response in 11 patients. The pain, tenderness, and dermatitis were evident by twenty-four hours and lasted approximately three days. Since all injections were given in areas where the regional nodes had not been removed, there was evidence of mild lymphadenopathy after the first three injections.

In this trial, four patients failed to recur for a longer period than the 9.7 months mean and 5 months median interval expected for previously treated recurrent stage II and stage III (metastases to skin only) melanoma, from Amer et al., who reported on 300 melanoma patients, at different stages of disease, that were referred to Wayne State University from 1965 to 1975 (24). The information from this paper served as the historical control for the VMO study. Therefore, since the mean survival of 4 of the patients was 21.5 months, one could postulate some effect of oncolysate therapy. Moreover, all of these responders to the VMO therapy had been treated previously with either DTIC, BCG, or both, after multiple surgical procedures with no apparent effect in either preventing recurrence or slowing disease progression until they received VMO therapy.

In the laboratory, only six of the twelve patients remained alive to provide sera at three months after the VMO treatment. All six pretreatment sera were negative in the assay. After 3 months of therapy, these six sera shared immunoreactivity in the SpA assay to one or more of the melanoma lines used in the oncolysate and in most cases recognized other human melanoma lines.

Moreover, posttreatment sera of the six patients who lived the longest in the trial showed definite binding to all six lines of melanoma target cells. Post-treatment sera of one other patient also showed binding to all melanoma target cells. Post-treatment sera of the other three patients also showed positive in the SpA assay, and therefore SpA assay became a test we used to assess the degree of immunostimulation by the VMO injections.

Work by Wallack et al. at Washington University School of Medicine after the completion of the preliminary phase I French study and prior to the phase I SEG (Southeastern Cancer Study Group) VMO trial (MEL 81 147P) has direct bearing on the specificity of the SpA assay in monitoring the degree of immunoreactivity in patients treated with the 1 mg dose of VMO. In May 1979, Wallack et al. started a pilot study at Washington University of St. Louis in which postsurgical patients with high-risk stage I and stage II melanoma were given VMO for a period of two years (injections were given intradermally once a week for 13 weeks and once every other week for 2 years). Sera was obtained from the patients prior to treatment and then every 3 months during the treatment. The target melanoma cell for the SpA assay in this trial was the line APO 27 obtained from Naval Bio-Science, Oakland, California. The sera from this trial was also tested against T-24 bladder carcinoma cells obtained from Dr. Meenhard Herlyn, Wistar Institute, Philadelphia, PA, and the MRC-5 human fibroblast line. The data showed that the sera of the VMO treated patients was positive in the SpA assay while control sera and cells were for the most part negative. Thus, the sensitivity of the assay at the 1 mg VMO dose was considered to be quite good and seemed to be a reasonable indicator of VMO induced immunoreactivity as compared to controls.

The discovery of the post-treatment serologic activity in the SpA assay made it possible to investigate the degree of VMO immunostimulation at different dose levels and schedules so that an optimal dose could be chosen for a definitive randomized trial. As a result, SEG MEL 81 147P was designed and opened in July 1982 for patient accrual. SEG MEL 81 147P was an FDA-approved pilot study of surgical adjuvant immunotherapy comparing different doses of VMO in patients with high-risk stage I and stage II disease.

In this trial, cohorts of 8 patients were treated with different doses of VMO measured in milligrams of protein. Each cohort of 8 patients received either 0.05 mg, 0.1 mg, 0.5 gm, 1.0 mg, 1.5 mg, or 2.0 mg by either delayed or immediate treatment schedule. Each dose was given intradermally, equally divided among several injection sites near major lymph node drainage areas. All therapy continued for 12 months or until recurrence. The institutions participating in the study were University of Alabama, Washington University, University of Florida, Duke University, University of Virginia, and University of Miami.

By April 1983 all 48 patients were on study. There were 30 male and 18 female patients. The median age was 45 years, ranging from 14 to 71 years. Ten patients originally had stage I disease whereas 38 had stage II disease. To date, 24 patients have had recurrences, including 4 with stage I and 20 with stage II disease. The mean disease-free interval of these patients was 8 months (range 1 to 23 months). A total of 13 patients have died. Twenty-four patients (6 stage I, 18 stage II) remain free of disease, the mean disease-free interval being 22 months (range 17 to 28 months).

As previously stated, the SpA assay was used to determine the formation of an antimelanoma-associated antibody from therapy with the VMO. Patients receiving delayed treatment had less SpA reactivity than patients receiving immediate treatment. In the immediate treatment arm, by 3 months, sera from 9 of 19 patients were positive and by 6 months, sera from 13 of 23 patients tested were positive. Two of the 4 patients at the 2 mg dose had positive findings at 3 months and all 4 patients had positive SpA results by 6 months. As a result, the 2 mg dose of VMO was chosen for all future trials. Furthermore, no life-threatening reactions were reported at any dose.

Pretreatment and treatment serum samples were further screened for the presence of antibodies to human leukocytes antigens (HLA) with a standard microcytotoxicity technique. Screening of serum samples for the presence of such antibody failed to show formation of HLA antibodies secondary to VMO stimulation. In fact, there was no correlation between SpA reactivity and the minimal reactivity seen in the HLA screening. These results seemed to preclude the possibility that the SpA reactivity was related to anti-HLA antibodies. Furthermore, since patients with circulating antibodies reactive with melanoma continued to be injected with antigen, the development of antigen-antibody complexes presented a potential problem for interpretation of serologic data as well as for possible clinical sequelae. However, on the basis of the two assays performed to determine circulating immune complex (CIC) levels, no meaningful evidence for elevation of immune complexes as a result of VMO stimulation was obtained.

To summarize the SEG 147P trial, immunotherapy with VMO as an adjuvant to surgery appears to be a promising direction for future management of melanoma. This trial demonstrated that toxicity is minimal and that VMO, especially at the 2-mg dose, stimulates immunoreactivity, as demonstrated by the SpA assay. Lastly, this reactivity is not HLA-related, nor is there evidence of immune complex formation.

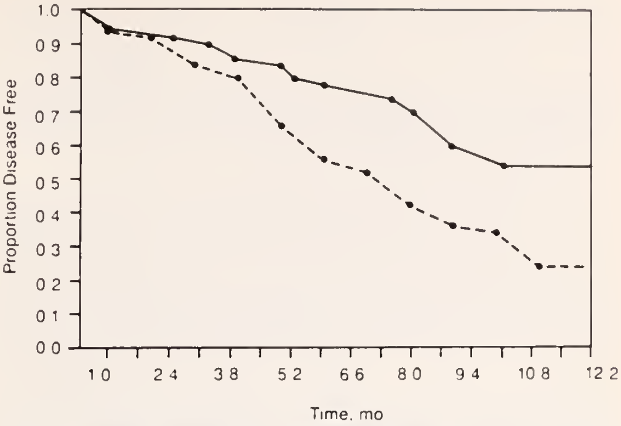


Fig. Disease-free survival of patients treated with vaccinia melanoma oncolysate (n = 39, solid line) compared with matched controls (n = 39, broken line) ($p = 0.04$).

Based on the results of this trial, the protocol for the SEG Phase II trial (MS 102), initiated in December 1984 and completed in August 1985, was the 2-mg dose, immediate treatment regimen. Of the 39 patients, 23 were male and 16 female. The median age was 44 years. One patient had stage I disease and 38 had stage II disease. As of August 1987, 20 of the 39 patients have had recurrences; 19 of these 20 had stage II disease. To date, there are 19 patients with no evidence of disease and disease-free survival of all patients in this trial of VMO was significantly greater ($p = 0.04$) than in matched controls from previous trials with BCG (23) (Fig).

In the laboratory, none of the 39 patients' pretreatment sera were positive in the SpA assay for melanoma-binding antibody. After 3 months of treatment, however, 17 of 33 (51%) had become positive, and this percentage increased at each 3-month interval. Dose escalation had no effect on either antibody titer or clinical results.

To further document the serologic responses of the patients in this study, an enzyme-linked immunosorbant assay (ELISA) procedure was used to detect IgG and IgM antibodies reactive with melanoma lines. This assay proved to be more sensitive than the SpA assay. Moreover, when the ELISA reactivity of patient sera was tested against the melanoma lines used in the VMO preparation, sera from patients who had recurrences had lower activity than sera from patients who remained free of disease.

To monitor the development of anti-HLA antibodies in the serum of patients undergoing VMO treatment, pretreatment and treatment serum samples from 25 patients were screened for anti-HLA antibodies. We tested HLA A/B/C and HLA-DR/DQ reactivity. Although many samples showed broad, nonspecific cytolytic activity against target cells both before and during treatment, none of the treatment sera demonstrated specific HLA-DR/DQ antibodies, and only one showed selective anti-HLA A/B reactivity against one of the cells in the panel.

The MS 102 study showed the VMO to benefit patients with stage II melanoma as compared to matched controls. Moreover, it showed that patients who had a high degree of activity in the ELISA assay for both IgG and IGM antibodies remained free of disease. Finally, the protocol showed that the antibodies generated were not HLA related. Therefore, the results of this study provide evidence that the VMO treatment may extend the disease-free survival of high risk melanoma patients in the adjuvant setting.

More recent work in Australia has provided further support for the efficacy of viral oncolysate in adjuvant therapy of melanoma. Hersey et al. (25) used vaccinia melanoma cell lysate (VMCL), a biological similar to VMO, in 80 patients with stage II melanoma. Two-year follow-up on 39 patients revealed a significant improvement in survival rates by comparison with 151 historical, nonrandomized controls matched for number of nodes, thickness of primary lesion, and sex. These results with VMCL support our findings with VMO.

The final proof of the value of the VMO can only be determined by a randomized double-blind adjuvant trial. The results of the MS 102 trial have provided enough compelling evidence for funding by the National Cancer Institute for Phase III randomized, prospective, double-blind, multi-institutional trial to study VMO versus vaccinia virus vaccine in patients with resected stage II melanoma. This trial is currently under way.

Summary

Based on antitumor effects observed with vaccinia virus infected tumor cell lysate in animal models, adjuvant immunotherapeutic clinical trials were undertaken in patients with melanoma using vaccinia virus infected melanoma oncolysate (VMO). Preliminary clinical trials showed that the VMO is safe except minimal side effects such as mild fever, pain, and tenderness at the site of VMO injection, and mild lymphadenopathy. The effective dose of VMO was investigated in a following trial using 0.05 to 2.0 mg doses of VMO. Clinical responses and laboratory monitoring of melanoma-specific antibody responses decided the 2 mg dose of VMO is optimal for future trials. In all the clinical trials, patients showed moderate responses and their postimmune sera contained melanoma-specific antibodies. In a Phase II clinical trial completed August 1985 19 of 39 stage II patients had a disease-free mean survival time of 24.6 months, statistically significant compared with historical controls. Because of compelling evidence of significant clinical responses in patients treated with VMO adjuvant immunotherapy in the Phase II trial, a prospective randomized multi-institutional double-blind Phase III adjuvant VMO immunotherapeutic trial using adjuvant therapy of vaccinia virus alone as control, was recently initiated. Results of this trial are anxiously anticipated.

References

1. Eilber FR, Nizze JA, Morton DL. Sequential evaluation of general immune competence in cancer patients: correlation with clinical course. Cancer 1975; 35:660.
2. Mitchison MA. Immunologic approach to cancer. Transplant Proc 1970; 2:92–103.
3. Sharpless GR, Davies MC, Cox HR. Antagonistic action of certain neurotropic viruses toward a lymphoid tumor in chickens with resulting immunity. Proc Soc Exp Bio Med 1950; 73:270.
4. Koprowski H, Love R, Koprowski I. Enhancement of susceptibility to viruses in neoplastic tissues. Texas Rep Biol Med 1957; 15:559.
5. Lindemann J, Klein P. Viral oncolysis: increased immunogenicity of host cell antigen associated with influenza virus. J Exp Med 1967; 126:93.
6. Lindermann J. Viruses as immunological adjuvants in cancer. Acta Biochim Biophys Acad Sci Hung 1974; 355:79.
7. Sauter C, Cavalli F, Lindermann J, et al. Viral Oncolysis: its application in maintenance treatment of acute myelogenous leukemia. In: Immunotherapy of Cancer: present status of trials in man. New York: Raven Press, 1978:355–363.
8. Greene A, Pratt C, Webster R, Smith K. Immunotherapy of osteosarcoma patients with virus-modified tumor cells. Ann NY Acad Sci 1976; 277:396.
9. Sinkovics J: Immunotherapy with viral oncolysates for sarcoma. JAMA 1977; 237:869.
10. Murray DR, Cassel W, Torbin A, Olkawski Z, Moore M. Viral oncolysates in the management of malignant melanoma. Cancer 1977; 40:680.
11. Cassel WA, Murray DR, Philips HS. A phase II study of the postsurgical management of stage II malignant melanoma with Newcastle disease virus oncolysate. Cancer 1983; 52:856–860.
12. Wallack MK, Steplewski A, Koprowski HK, Rosate EF, George Jr, Hullihan B, Johnson J. A new approach in specific, active immunotherapy. Cancer 1977; 39:560–564.
13. Zingernagel RM, Althage A. Antiviral protection by virus immune cytotoxic T cells: infected target cells are lysed before infectious progeny is assembled. J Exp Med 1977; 145:644.
14. Hapel AJ, Bablanian R, Cole GA. Inductive requirements for the generation of virus-specific T lymphocytes: 1. The nature of the host cell-virus interaction that trig-

gers secondary pox virus-specific cytotoxic T lymphocyte induction. J Immunol 1978; 121:736.

15. Senik A, Newport-Sautes C. Association between H-2 and vaccinia virus-induced antigens on the surface of infected cells. J Immunol 1976; 6:679.
16. Koszinowski U, Eitt H. Role of early viral surface antigens in cellular immune response to vaccinia virus. Eur J Immunol 1976; 6:679.
17. Wallack MK. Specific tumor immunity produced by the injection of vaccinia viral oncolysates. J Surg Res 1982; 33:11–16.
18. Wallack MK. Specific immunotherapy with vaccinia oncolysates. Cancer Immunol Immunother 1981; 12:1–4.
19. Leibovitz A, Stinson J, McComb WB, McCoy C, Mazur K, Mabry M. Classification of human colorectal adenocarcinoma cell lines. Cancer Res 1976; 36:4562–4569.
20. Noel P, Falcon M, Leftheriotis E, et al. Interet de la morphologie ultrastructurale dans la preparation des oncolysats de melanome malin humain. Ann Anat Pathol 1979; 24:125–136.
21. Dunn D, Barth R. Identification of melanoma cells by formaldehyde-induced fluorescence. Cancer 1975; 33: 701–706.
22. Haryln M, Clark WH, Mastrangelo M, et al. Specific immunoreactivity of hybridoma-secreted monoclonal antimelanoma antibodies to cultured cells and freshly derived human cells. Cancer Res 1980; 40:3602–3609.
23. Wallack MK, Bash JA, Leftheriotis E, et al. Positive relationship of clinical and serologic responses to vaccinia melanoma oncolysate. Arch Surg 1987; 122:1460–1463.
24. Amer MH, Al-Sarvaf M, Vaitkevicuis UK. Clinical presentation, natural history and prognostic factors in advanced melanoma. Surg Gynecol Obstet 1979; 149:168–192.
25. Hersey P, Edwards A, Coates A, Shaw H, McCarthy W, Milton G. Evidence that treatment with vaccinia melanoma cell lysates (VMCL) may improve survival of patients with stage II melanoma. Cancer Immunol Immunother 1987; 25:257–265.

Humoral Antimelanoma Immunity:

Induction by Mouse Antiidiotypic mAb, MK2-23 and Association with Survival of Patients with Advanced Melanoma

A. Mittelman, M.D., C. Puccio, M.D., H. Chun, M.D., and T. Ahmed, M.D.

Active specific immunotherapy in patients with solid malignant tumors aims at inducing or enhancing the immune response against antigens expressed on tumor cells with the expectation that this immune response will affect the biology of malignant cells and/or affect their destruction by immune modulation. Testing this hypothesis has been hampered by difficulties to purify sufficient amounts of immunologically functional human tumor associated antigens as immunogens for clinical trials. Hybridoma methodology which has provided the opportunity to prepare large amounts of antiidiotypic monoclonal antibodies with well defined and standarized characteristics. Antiidiotypic antibodies have features which mimic the corresponding antigen and induce immunity to it in recipients who have been exposed to the antigen (1) and did not develop the immunity to the antigen. In previous investigations we have shown that mouse antiidiotypic monoclonal antibody (mAb) MK2-23 bears the mirror image of the human high molecular weight-melanoma associated antigen (HMW-MAA) identified on human melanoma cells with mouse mAb (2). Administration of mouse mAb MK2-23 induces anti HMW-MAA immunity in about 60% of patients with stage IV melanoma (3). The immune response appears to be associated with increased survival of patients with metastatic melanoma (3).

The mAb MK2-23 recognizes an idiotope within the antigen combining site of the syngeneic anti HMW-MAA mAb 763.74 (2). The mAb MK2-23 induces anti HMW-MAA antibodies and delayed type hypersensitivity reaction to HMW-MAA bearing human melanoma cells in BALB/c mice (4). Furthermore, immunization with mAb MK2-23 can break tolerance to self HMW-MAA in xenogeneic hosts (5). Rabbits which express HMW-MAA in their normal tissues (6) produce anti HMW-MAA antibodies following immunization with mAb MK2-23 (5).

Sera were harvested on day 42 from rabbits immunized with mAb MK2-23 coupled to KLH and mixed with CFA on day 0, with IFA on day 14 and with PBS on day 28. Immune sera were absorbed with cultured B lymphoid cells L14 (4×10^7 cells/mL serum). Unabsorbed and absorbed sera (100uL) were incubated with melanoma cells M14/13 (2×10^5 cells/well) for 2 h at 4°C. Plates were then washed 3 times and added with ^{125}I-anti rabbit IgG + M xenoantibodies (1×10^5 cpm/well). At the end of an additional 2-h incubation at 4°C, plates were washed 5 times. Bound radioactivity was measured in a gamma counter. Results are expressed as bound cpm/well. Cultured B lymphoid cells L14, which are autologous to melanoma cells M14/13; preimmune serum; and serum from a rabbit immunized with the unrelated isotype matched anti-id mAb MF9-10 were used as specificity controls.

The sera from rabbits immunized with mAb MK2-23 displayed a higher reactivity with HMW-MAA bearing human melanoma cells M14/13 than with its autologous B lymphoid cells L14 (Fig. 1), which do not express HMW-MAA. Absorption of immune rabbit sera with cultured B lymphoid cells L14 removes the low reactivity with B lymphoid cells, but does not affect the reactivity

---

From the Department of Medicine, Division of Neoplastic Diseases, New York Medical College, Valhalla, NY. Address reprint requests to A. Mittelman, MD, Department of Medicine, New York Medical College, Munger Pavilion, Valhalla, NY 10595.

with cultured melanoma cells. Furthermore, antiantiidiotypic antibodies purified from immune rabbit sera by affinity chromatography on the immunizing mAb MK2-23 immunoprecipitate HMW-MAA from radiolabeled melanoma cells. The immunogenicity of mAb MK2-23 in syngeneic and xenogeneic hosts is markedly enhanced by its conjugation to keyhole limpet hemocyanin (KLH) and administration with an adjuvant.

Methods and Patients

A clinical trial approved by the IRB was implemented in 25 patients with stage IV melanoma; 2 mg of mAb MK2-23 conjugated to KLH and mixed with 0.1 ml of Bacillus Calmette-Guerin (BCG) were administered subcutaneously on day 0, 7, and 28. Additional injections were given if the titer of antiantiidiotypic antibodies was low at the 1:8 dilution in the fourth week of treatment, and whenever the titer decreased at the 1:8 dilution.

Sera (100 uL) were absorbed with cultured B lymphoid cells (4 × 10^6) by a 4-hr incubation at 4°C. Unabsorbed sera were added to cultured melanoma cells M14/13 and autologous cultured B lymphoid cells L14 in 96-well microtiter plates. At the end of 2-h incubation at 4°C, cells were washed three times with PBS and incubated with ^{125}I-anti human Ig xenoantibodies (1 × 10^5 cpm) for 2 h at 4°C. Plates were then washed five times with PBS. Bound radioactivity was measured in a gamma counter. Results were expressed as bound cpm/well.

Two of the 25 patients did not complete four weeks on treatment. Fourteen of the remaining 23 immunized patients developed humoral anti HMW-MAA immunity.

The anti HMW-MAA specificity of the antibodies induced in patients by the immunization with the antiidiotypic mAb MK2-23 is reflected by a higher reactivity of the patients' sera with cultured melanoma cells M14/13 than with autologous B lymphoid cells L14. Furthermore, absorption of sera with culture B lymphoid cells removed the reactivity with B lymphoid cells, but did not markedly affect that with cultured melanoma cells (Fig. 2).

Lastly, antiantiidiotypic antibodies purified from patient sera by affinity chromatography on immunizing antiidiotypic mAb MK2-23 immunoprecipitated two components with the same electrophoretic mobility as the two components immunoprecipitated by mAb 763.74 from an NP40 extract of cultured melanoma cells Colo 38 (3).

Sera were obtained on day 126, 133, 140, 147, 154, 161, 168, 175, 182, and 190 from patients immunized on day 0, 7, 28, and 112 with subcutaneous injections of mAb MK2-23 (2 mg/injection) conjugated to KLH and mixed with BCG. Sera were pooled and utilized to purify antiantiidiotypic antibodies by affinity chromatography on mAb MK2-23 following removal of anti-mouse Ig antibodies. Cultured melanoma cells were labeled with ^{125}I and solubilized with NP40. The extract was then immunoprecipitated with unsolubilized antiantiidiotypic antibodies. Antigens were then eluted from the immunoadsorbent and analyzed by SDS-PAGE in a 5% slab gel in the presence of 2% β-mercaptoethanol. Gels were pro-

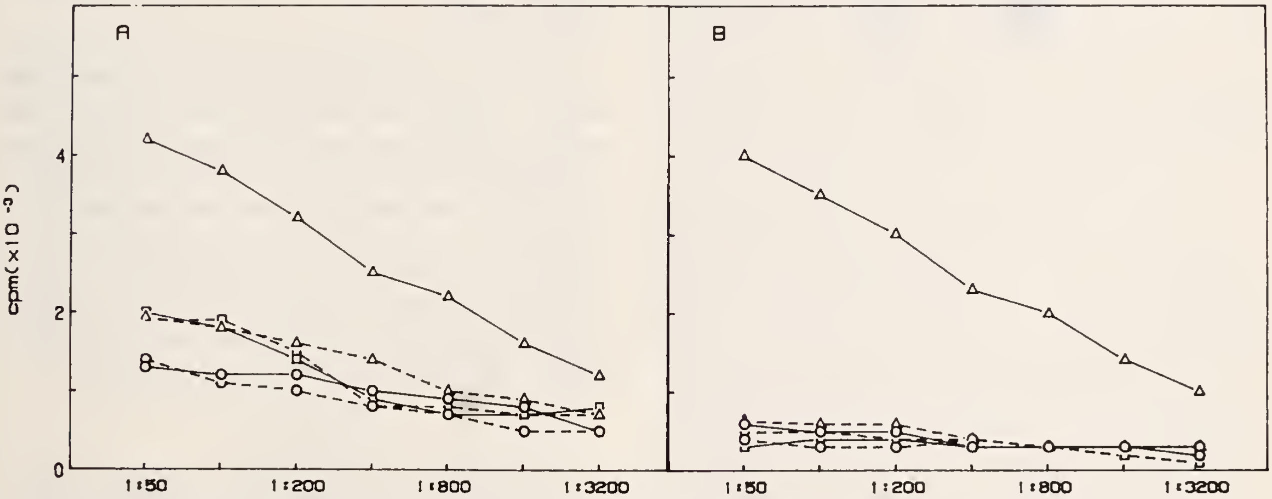


Fig. 1. Effect of absorption (panel A, unabsorbed; panel B, absorbed sera) with cultured human B lymphoid cells L14 (– – –) on differential reactivity with cultured human melanoma cells M14/13 and autologous B lymphoid cells L14 of sera from rabbits immunized with mouse anti-id mAb MK2-23. Results in bound cpm/well; cultured B lymphoid cells L14, preimmune serum (○), and serum from rabbit immunized with unrelated isotype matched anti-id mAb MF9-10 (□) used as specificity controls.

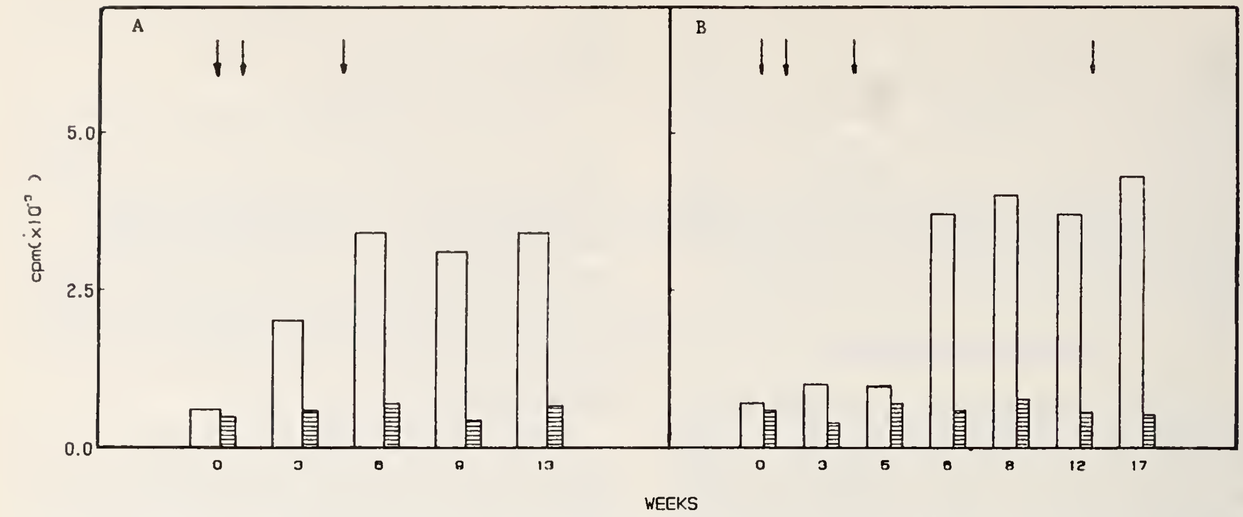


Fig. 2. Differential reactivity with cultured human melanoma cells M14/13 (white bars) and autologous cultured human B-lymphoid cells L14 (hatched bars) of sera from patients immunized with mouse antiidiotypic mAb MK2-23 (panel A, patient AK; panel B, patient AM). Arrows, days of immunization; results expressed as bound cpm/well.

cessed for autoradiography using a Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY).

Anti HMW-MAA mAb 763.74 and anti mouse Ig antibodies purified from patient serum by affinity chromatography on mouse IgGl mAb were used as controls (Fig. 3).

The antibodies induced by mAb MK2-23 recognize the same (or spatially close) determinant(s) that is defined by anti HMW-MAA mAb 763.74, since the latter do not react with cultured melanoma cells M14/13 coated with sera from immunized patients. Furthermore, patients' sera do not react with purified HMW-MAA bound to mAb 763.74 coated microtiter plates. Patients' antibodies express the idiotope recognized by mAb MK2-23 in their antigen combining sites, since mAb MK2-23 inhibits the binding of patients' sera to cultured human melanoma cells.

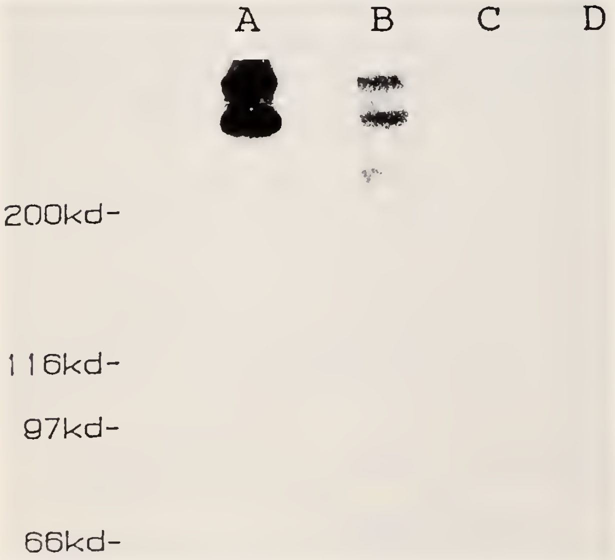


Fig. 3. SDS-PAGE analysis of antigens immunoprecipitated from radiolabeled melanoma cells Colo 38 by antiantiidiotypic antibodies purified from serum from patient immunized with mouse antiidiotypic mAb MK2-23. Migration of m.w. standards indicated on left.

Results

The induction of human anti HMW-MAA immunity in patients with melanoma was associated with a significant ($p < 0.05$) prolongation of their survival: the median survival of the 14 patients who had developed humoral anti HMW-MAA immunity was 33 weeks, while that of the 9 without detectable anti HMW-MAA antibodies was 19 weeks.

In spite of an increase in the level of human antimouse Ig antibodies, patients experienced neither allergic nor anaphylactic reactions. Repeated administrations of mouse antiidiotypic mAb MK2-23 were associated with local toxicity. The latter was caused by the administration of BCG and consisted of erythema, induration and ulceration at the sites of immunizations. Occasionally, patients developed flulike symptoms and complained of arthralgias and myalgias, which were controlled with acetoaminophen.

Summary

This study has shown that a mouse antiidiotypic mAb bearing a mirror image of HMW-MAA can break tolerance to a well-defined self HMW-MAA and can induce humoral anti HMW-MAA immunity in patients with melanoma. Furthermore, the induction of an immune response to a well-defined melanoma-associated antigen has been shown to be associated with a favorable clinical response. If this association reflects a cause-effect relationship between the two parameters, the anti HMW-MAA immunity induced by mouse antiidiotypic mAb MK2-23 may effect immune destruction of melanoma cells and may interfere with the metastatic potential of those cells because of the suggested role of HMW-MAA in this phenomenon.

References

1. Mittelman A, Chen ZJ, Kageshita T, et al. Active specific immunotherapy in patient with melanoma: a clinical trial with murine anti-idiotypic monoclonal antibodies elicited with syngeneic anti-high molecular weight—melanoma associated antigen (HMW-MAA) monoclonal antibodies. J Clin Invest 1990; 86:2136–2144.
2. Kusama M, Kageshita T, Chen ZJ, Ferrone S. Characterization of syngeneic and anti-idiotypic monoclonal antibodies to murine anti-human molecular weight melanoma-associated antigen monoclonal antibodies. J Immunol 1989; 143:3844–3852.
3. Mittelman A, Chen ZJ, Yang H, Wong GY, Ferrone S. Human high molecular weight-melanoma associated antigen (HMW-MAA) mimicry by mouse anti-idiotypic mAb MK2-23: induction of humoral anti HMW-MAA immunity and prolongation of survival in patients with stage IV melanoma. Proc Natl Acad Sci USA 1992;89:466–470.
4. Chen ZJ, Yang H, Ferrone S. Human high molecular weight-melanoma associated antigen mimicry by mouse antiidiotypic mAb MK2-23: characterization of the immunogenicity in syngeneic hosts. J Immunol 1991; 147:1082–1090.
5. Chen ZJ, Yang H, Liu CC, Hirai S, Ferrone S. Human high molecular weight-melanoma associated antigen (HMW-MAA) mimicry by mouse antiidiotypic mAb MK2-23: induction of humoral anti HMW-MAA immunity in xenogeneic hosts with a constitutive HMW-MAA expression. 1991.
6. Schlingemann RO, Rietveld FJR, De Waal RMW, Ferrone S, Ruiter DJ. Expression of the high molecular weight melanoma-associated antigen by epricytes during angiogenesis in tumors and in healing wounds. Am J Pathol 1990; 136:1393–1405.

Immunotherapy of Metastatic Melanoma with Interleukin-2

Paolo Alberto Paciucci, M.D.

Although resistant to chemotherapy and radiation therapy, melanoma in its early stages is responsive to immunologic manipulation in immunocompetent patients. Effective forms of treatment do not exist, however, for tumors that recur following resection.

Interleukin-2 (IL2), initially described in 1976 as a factor in the supernatant of lectin-activated lymphocyte cultures that supported the growth of activated T cells (1), was shown by further characterization to be capable of inducing the generation of cell-mediated cytotoxic mechanisms against autologous and allogeneic tumor cells (2–5). The premise for antineoplastic activity was confirmed in several preclinical murine systems (6–8). With the availability of recombinant product, immunotherapy models with IL2 were rapidly translated to the clinic in order to evaluate the role of the lymphokine and of lymphokine-activated tumor killer cells in the therapy of cancer. The first phase I trial of immunotherapy with IL2 at the Mount Sinai Hospital was initiated in 1985.

Based on the observation that IL2 had a short half-life when injected by intravenous bolus (9), we at The Jeanne B. Lambert Laboratory for Cancer Research, elected to investigate the activity of IL2 administered by constant intravenous infusion. These studies (10–12) showed similar anticancer activity and a somewhat decreased incidence of dose-limiting toxic side effects when compared to those reported for high-dose, bolus IL2 (13–15).

Here I report our experience in the treatment of metastatic malignant melanoma with 3 consecutive trials of constant-infusion IL2 plus adoptive cell transfer, IL2 only, and IL2 plus chemotherapy.

Treatment Methods

To be eligible to enter each study, patients had to have histologically documented evidence of melanoma and metastatic disease had to be measurable by physical examination or standard radiological techniques. Patients also had to have a performance status of 0–2 by the Cancer and Leukemia Group B (CALGB) criteria (16), have no active viral or bacterial infections, have had no chemotherapy for the previous 4 weeks, and not be candidates for surgical or radiotherapeutic procedures. An indwelling catheter was surgically positioned at the time of entry on study. IRB approval was given, and informed consent was obtained from all patients according to institutional and federal guidelines.

All patients were closely monitored for treatment-induced toxicities; vital signs were taken several times a day, often hourly or more frequently, as needed. Body weight and fluid balance were followed daily. Serum chemistries, peripheral blood counts, and differentials were monitored several times a week; determinations were done daily when mandated by treatment-induced toxicities. Each patient's immunologic profile, consisting of the determination of immune surface markers, spontaneous and IL2-induced augmentation of tumor cytotoxicity in vitro, was studied at baseline and weekly thereafter.

Response criteria were as follows: complete response was defined by the complete disappearance of all measurable lesions; partial response

---

From the The Jeanne B. Lambert Laboratory for Cancer Research and the Department of Neoplastic Diseases, Mount Sinai School of Medicine. Address reprint requests to the author, who is Associate Professor of Neoplastic Diseases, Mount Sinai School of Medicine, at Box 1129, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

was defined as a decrease equal to, or greater than, 50% of the product of maximum perpendicular measurements of metastatic lesions; minor response was defined as a decrease in the product of perpendicular measurements of tumors between 25 and 50%; stable disease was defined as less than 25% change of tumor measurements. All responses had to last a minimum of 4 weeks. Progressive disease was defined as an increase of tumor lesions greater than 25% or the appearance of new lesions in a patient with otherwise regressing tumors.

Study 1. The first trial of immunotherapy with IL2 was an initial phase I evaluation of the lymphokine administered together with adoptive transfer of autologous lymphocytes activated in vitro with the lymphokine. Recombinant, des-alanyl, serine-125 interleukin-2 (IL2) was generously provided by Cetus Corporation (Emeryville, CA) and was given by constant intravenous infusion for 6 consecutive days/week for 4 consecutive weeks. Study patients underwent leukopheresis once weekly immediately after the lymphokine infusion was discontinued. In vitro activation was carried out at 37°C for 16 hours in a closed-system culture using conventional blood bank gas-permeable transfer packs. Mononuclear cells were separated from platelets and red cells by spontaneous sedimentation and were diluted in serum-free, human albumin supplemented culture medium (RPMI 1640) to a concentration of 2×10^6 cells/ml. IL2 was added at the concentration of 1000 units/ml. Prior to reinfusion, cells were washed, filtered, assayed for cytotoxicity against tumor cells in vitro and for contamination.

Infusions of IL2 were restarted immediately after cell transfer. IL2 doses were escalated in consecutive cohorts of patients from 1 to 1.5, 2.3, 3.4, and 7.5 million units/m^2. Additionally, in each cohort, doses were incremented by 50%, as individually tolerated, in order to define the maximally tolerated dose. Of 25 patients with different types of cancer who entered this study, 7 had a diagnosis of melanoma.

Study 2. The second study was a phase II evaluation of IL2 only. The adoptive cell transfer was omitted based on the evidence, gathered from the preceding phase I study, that IL2 possesses direct lymphoproliferative and antitumoral effects and that the cell transfer did not add to the activity of the regimen while contributing to its toxicity.

Patients in this study were all started at the same dose of Cetus IL2 (3.4 million units/m^2 daily for 6 days). Weekly dose increments were made, as individually tolerated in each patient. Treatment was stopped on the appearance of limiting toxicities and was resumed on their resolution. A total of 41 patients, of whom 12 had melanoma, were entered in this study.

Study 3. The third study is an ongoing evaluation of IL2 combined with chemotherapy and constitutes a development of the previous two studies.

The chemotherapy regimen of lomustine, constant-infusion bleomycin, and cisplatin was empirically used, and found to be active, in those patients with melanoma who had either progressed on, or relapsed from, treatment with IL2 in the previous two trials.

The lymphokine for this study is generously provided by Hoffman-La Roche Inc. (Nutley, NJ). This recombinant molecule is structurally similar to natural IL2, from which it differs by a methionine residue at the N terminus.

IL2 is administered for only 5 days (Monday to Friday) for only two consecutive weeks. This modification is based on the observation in the preceding trials that maximal augmentation of antitumor, cell-mediated immune mechanisms is achieved within the first 2 weeks of treatment and that further administration of IL2 does not upregulate antitumor cytotoxicity, while it does substantially add to toxicity (17–18).

In addition, in order to explore the activity and toxicity of different therapeutic schedules, patients are randomized to receive the same dose on the same time schedule of IL2 by either constant intravenous infusion or by bolus subcutaneous injection. Ten patients in this study are currently evaluable for response.

TABLE

Response to Treatment

Study	n	CR	PR	SD	PD
1	6	0	1	2	3
2	12	2	5	0	5
3	10	2	2	0	6
Total dose IL2*	N	Responses	Dose Intensity†	N	Responses
<50	9	0	<10	7	2
50–100	8	4	20–30	8	4
>100	11	8	>30	13	6

N, patients entered in each study; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

* Total dose of IL2 over entire treatment period in million units/m^2.

† Total dose of IL2 in million units/m^2, divided by duration of treatment (weeks).

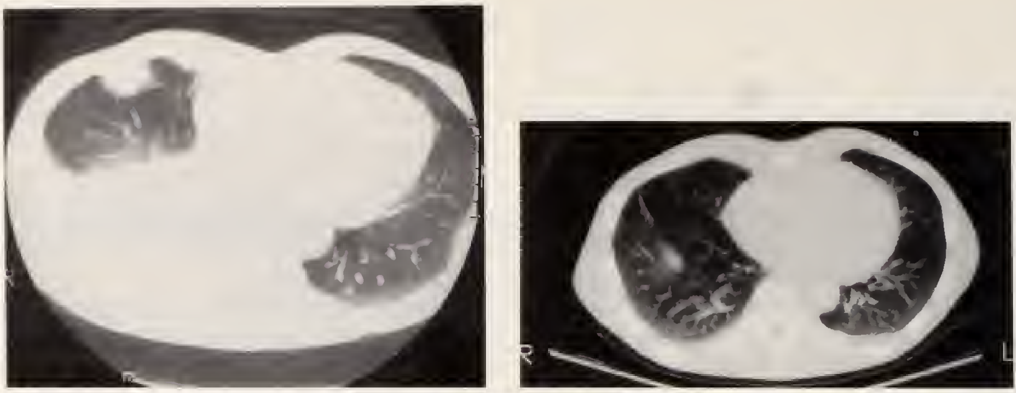


Fig. 1. Chest CT scan showing 2.5 × 2.5 cm pleural melanoma metastasis on anterior right chest wall before (left) and after (right) immunochemotherapy.

Results

A total of 28 patients were entered into 3 different trials of immunotherapy with IL2. Of these, 23 were males, 5 females. Their age ranged from 26 to 71, the median age being 50. In 7 patients the diagnosis of metastatic disease was made without antecedent history of melanoma. The most frequently involved sites were draining or distant lymph nodes (14 patients), lung (10 patients), liver (9 patients), subcutaneous sites (6 patients), skin (5 patients), bone (4 patients), gastrointestinal (1 patient). Fifteen patients had involvement of one site, 12 of two, and 3 patients of more than 2 sites. Treatment toxicities were substantial and they have already been detailed elsewhere (12, 18). Three patients (one with evidence of response) were taken off study for dose-limiting toxicity; three patients did not complete the treatment plan because of rapid disease progression. In the last study, one patient randomized to the subcutaneous bolus regimen was treated on an ambulatory basis.

In the phase I, dose-searching study of IL2, only 1 patient had regression of metastatic disease (Table). Notably, he was the only one of the six patients in that study who was entered at doses of IL2 that proved to have antitumoral activity.

In the second study, IL2 only, all patients were started at the same dose of IL2. Of 12 patients, 2 had complete regression, 5 had partial response and 5 had progressive disease.

Of the 10 patients entered in the immunochemotherapy study, 4 responded, 2 completely and 2 partial. Response occurred in both treatment arms. Six patients had progressive disease.

Responses were short-lived in all 3 studies and lasted an average of only 4 months (range 1–7 months); only one patient has a continuing response at 5 months on study.

Total Dose and Dose Intensity. As already reported in previous studies, which involved larger numbers of patients with many different neoplastic conditions and other types of malignancies, a direct relationship exists between tumor regression and total dose of IL2. This relationship is also present when only patients with melanoma are considered.

The relationship between dose intensity (the ratio between total dose of IL2 and total duration of treatment, in weeks) and response, however, is not linear, and the response rate occurring at intensities between 20 and 30 is higher, though not significantly, than those at intensities over 30.

The antitumoral activity of the regimen used in the third study in two patients is illustrated (Figs. 1, 2).

Due to the small size of the sample definitive conclusions cannot be derived on the implication of the skewed relationship between response and IL2 dose intensity. It is legitimate to assume, however, that lack of direct relationship between tumor response and dose intensity must be indicative of the differences in antitumoral mechanisms of biological therapies and chemotherapy, a treatment modality in which tumor cell kill is often a strict function of dose intensity.

Prognostic Factors. We and others have attempted to define prognostic factors for response in patients undergoing treatment with biologicals; it is generally reputed that the magnitude of lymphoproliferative effects induced by the lymphokine is a function of the host's "immunobiological status" and that this is the chief determinant for response. In vivo proliferation of lymphocytes after the first week of therapy is

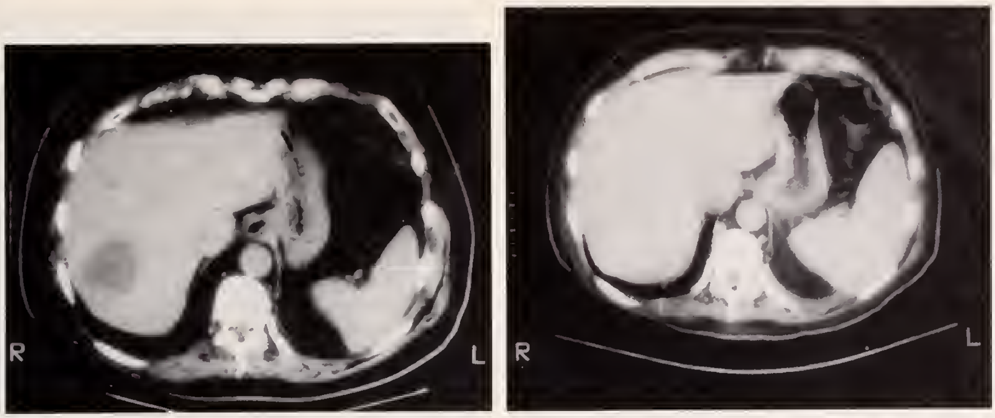


Fig. 2. Abdominal contrast CT scan showing 3 × 4 cm hepatic lesion from metastatic melanoma before (left) and after (right) immunochemotherapy.

substantially higher in patients who subsequently responded than in those who did not (10, 12). In the present group of patients, the baseline average lymphocyte count of nonresponders and responders was identical (1,504/μL vs. 1,685/μL, respectively), but after the first week of treatment, rebound of lymphocytes in the peripheral blood was substantially different in the two groups (4,730/μL in nonresponders and 10,000/μL in responders, $p = .051$). When the data are normalized to allow comparison between patients who received comparable dose-intensities of IL2, lymphoproliferative effects are still higher in responders, but the difference from nonresponders loses statistical significance.

A further attempt to define what constitutes the immunobiological status is the correlation between clinical response to IL2 and in vitro quantitation of lymphokine-activated killer cell activity. In this respect, we recently reported that patients who responded to IL2 had higher lymphokine-activated cytotoxic activity than those who did not respond (19). When the same analysis is applied to patients with melanoma, the average lytic units (defined as the number of effector cells in each effector population necessary to cause the lysis of 20% of target cells) determined after the first week of treatment was 5.2 in nonresponders and 17.3 in responders. This difference, though suggesting a role for lymphokine-activated killer cells in antitumor responses, is not significant. The effects of cytolytic mechanisms causing regression of tumors during IL2 therapy are shown in Fig. 3.

Although the progressive, IL2-driven increase of native tumor lymphocyte infiltrates strongly supports the contention that cell-mediated mechanisms are relevant for clinical tumor regression, lymphocyte proliferation and activation may not be absolutely necessary and indispensable conditions for the achievement of response. Recent hypotheses have involved other mechanisms, such as local or systemic IL2-induced secondary release of other lymphokines (TNF, interferons). A better understanding is needed of the relationship between these lymphokines, the ability of IL2 to trigger their release, and their direct (or indirect) antitumoral activity. Until such understanding is gained it may not be possible to define exactly what constitutes host “immunobiological status” and what factors allow or preclude tumor regression in response to therapy with biologicals.

At present the further development of cancer immunotherapy with IL2 hinges on success in the search for optimal, less toxic (but similarly active) schedules of treatment. These goals may be reached through the identification of drugs or procedures that effectively control or prevent the clinical toxicity of IL2 without precluding its antineoplastic activity. Such studies are currently under way, and their clinical application may take place in the not too distant future. If treatment with IL2 is made more tolerable, it may be possible not only to explore whether high-dose regimens yield better quality responses, but also to extend adjuvant immunotherapy to patients immediately after surgical resection of primary, high-risk lesions. Because prolonged and costly hospitalization is necessary for the administration of active antitumoral doses of IL2, and tumor responsiveness is still unpredictable, such a proposition is currently unacceptable for the tens of thousand of patients in the U.S. alone who are annually diagnosed as having cutaneous melanoma.

On the available evidence that, at this stage of minimal residual disease, melanoma is suscep-

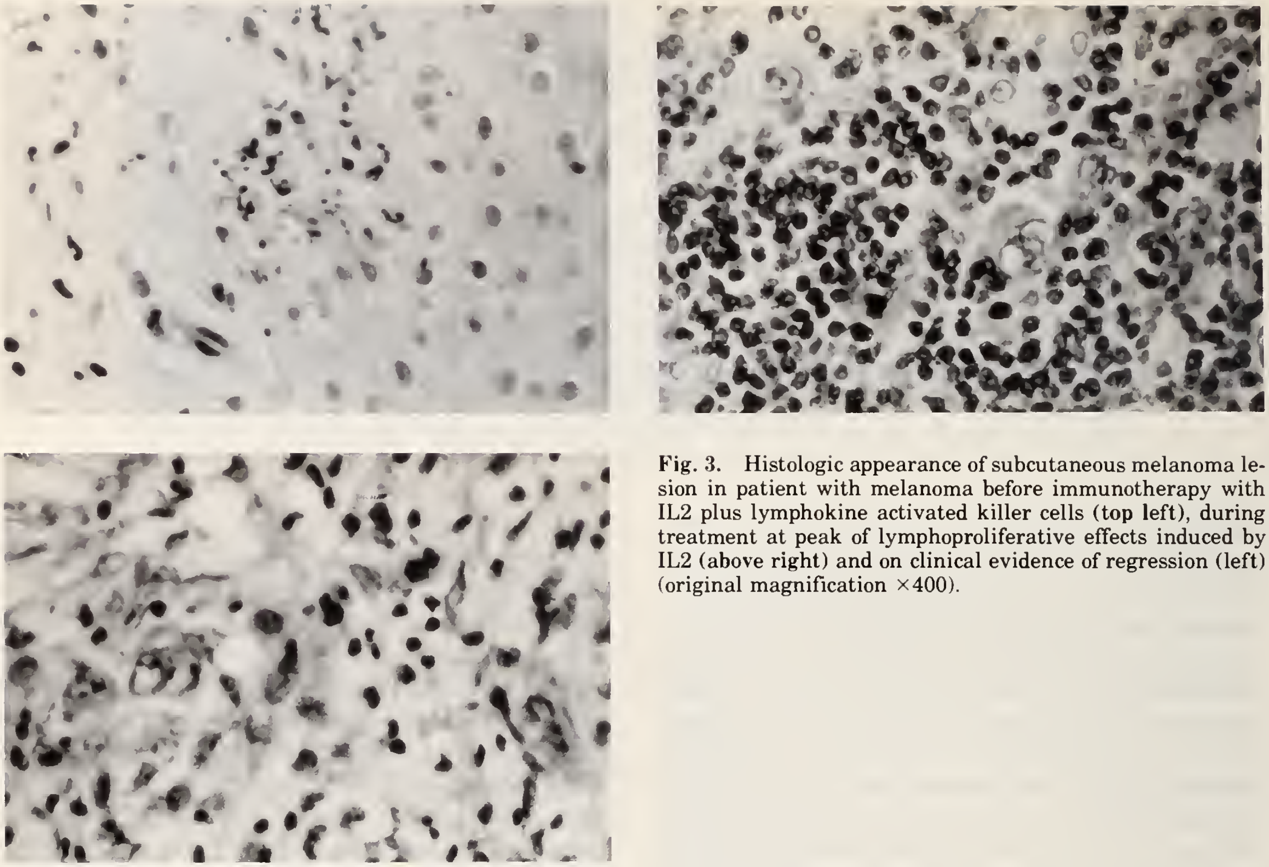


Fig. 3. Histologic appearance of subcutaneous melanoma lesion in patient with melanoma before immunotherapy with IL2 plus lymphokine activated killer cells (top left), during treatment at peak of lymphoproliferative effects induced by IL2 (above right) and on clinical evidence of regression (left) (original magnification ×400).

tible to eradication by immune mechanisms, it is possible that the greatest impact of IL2 in the therapy of cancer will be its ability to prevent recurrence of disease rather than to induce permanent regression of established metastases.

References

1. Morgan DA, Ruscetti FW, Gallo RC. Selective in vitro growth of T-lymphocytes from normal bone marrow. Science 1976; 193:1007–1008.
2. Kuribayashi K, Gillis S, Kern D, Henney CS. Effects of interleukin-2 and interferon on cell growth and cytotoxic reactivity. J Immunol 1981; 126:2321–2327.
3. Yron I, Wood TA, Spiess PJ, Rosenberg SA. In vitro growth of murine T-cells. 5. The isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. J Immunol 1981; 125:238–245.
4. Grimm EA, Mazumder A, Zhang HG, Rosenberg SA. Lymphokine activated killer-cell phenomenon: lysis of natural-killer resistant fresh solid tumor cells by interleukin-2 activated autologous human peripheral blood lymphocytes. J Exp Med 1983; 155:1823–1841.
5. Wagner H, Hardt C, Heeg K, Rollinghoff M, Pfizenmaier K. T-cells derived helper factor allows in vivo induction of cytotoxic T-cells in nu/nu mice. Nature 1980; 284:278–280.
6. Merluzzi VJ, Kenney RE, Schmid FA, Choi YS, Foanes RB. Recovery of the in vivo cytotoxic T-cell response in cyclophosphamide treated mice by injection mixed lymphocyte culture supernatants. Cancer Res 1981; 41:3663–3665.
7. Cheever MA, Greenberg PD, Fefer A, Gillis S. Augmentation of the anti-tumor therapeutic efficacy of long-term cultures T-lymphocytes by in vivo administration of purified interleukin-2. J Exp Med 1982; 155:968–980.
8. Mazumder A, Rosenberg SA. Successful immunotherapy of natural killer cell resistant established pulmonary melanoma metastases by the intravenous adoptive transfer of syngeneic lymphocytes activated in vitro by interleukin-2. J Exp Med 1984; 159:495–507.
9. Lotze, Matory YL, Ettinghausen SE, Rayner AA, et al. In vivo administration of purified interleukin-2. II. Half-life, immunologic effects and expansion of peripheral lymphoid cells in vivo with recombinant interleukin-2. J Immunol 1985; 135:2865–2875.
10. West WH, Tauer KW, Yannelli JR, et al. Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. N Engl J Med 1987; 316:898–905.
11. Sosman JA, Kohler PC, Hank J, et al. Repetitive weekly cycles of recombinant interleukin-2: responses of renal cell carcinoma with acceptable toxicity. J Natl Cancer Inst 1988; 80:60–63
12. Paciucci PA, Holland JF, Glidewell O, et al. Recombinant Interleukin-2 (rIL2) by continuous infusion and adoptive transfer of rIL2 activated cells in patients with advanced cancer. J Clin Oncol 1989; 7:869–878.
13. Rosenberg SA, Lotze MT, Muul LM, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 1985; 313:1484–1492.
14. Rosenberg SA, Lotze MT, Muul LM, et al. A progress re-

port on the treatment of 157 patients with advanced cancer using lymphokine activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 1987; 316:879–897.

15. Fisher RI, Coltman CA, Doroshow JH, et al. Metastatic renal cell cancer treated with interleukin-2 and lymphokine-activated killer cells. Ann Intern Med 1987; 108:518–523.
16. Kung FH, Nyhan WL, Cuttner J, et al. Vincristine, prednisone and L-asparaginase in the induction of remission of children with acute lymphoblastic leukemia following relapse. Cancer 1978; 41:420–434.
17. Paciucci PA, Holland JF, Ryder JS, et al. Immunotherapy with interleukin-2 by constant infusion with and without adoptive cell transfer and weekly doxorubicin. Cancer Treat Rev 1989; 16(suppl):67–81.
18. Paciucci PA, Bekesi JG, Ryder JS, Odchimar R, Chahinian PA, Holland JF. Immunotherapy with IL2 by constant infusion and weekly doxorubicin. Am J Clin Oncol 1991; 14:341–348.
19. Paciucci PA. Anti-tumor effects of interleukin-2. In: Indiveri F, Puppo F, Scudelletti M, Esculapio ED, eds. Biological response modifiers: applications in clinical medicine. San Remo, 1990, 145–153.

Immunoregulation, Immune Defects, and Clinical Strategies in HIV Infection

H. Clifford Lane, M.D.

In this discussion I will attempt to illustrate ways in which studies of the immune system have helped us understand and deal more effectively with some of the clinical manifestations of HIV infection. In general the immune systems of patients with HIV infection are characterized by a decrease in the number and function of helper/inducer (CD4) T lymphocytes. This is the hallmark of the immunologic defect in HIV infection. However, a variety of other immunologic abnormalities are seen. Among the more important defects, in addition to the decline in CD4 T cells, are:

- An increase in the number of suppressor-cytotoxic (CD8) T lymphocytes paradoxically coupled with a decrease in cytotoxic T-cell function. This decrease is presumably due to a lack of CD4 cell induction.
- A decrease in natural killer cell function. This is similar to the decrease in cytotoxic T-cell function. It occurs in the face of normal numbers of NK cells and again is probably due to a lack of the inductive signals provided by the CD4 T lymphocyte.
- A polyclonal B-cell activation with elevated levels of immunoglobulin. This is most likely due to Epstein-Barr virus activation of these cells in the absence of the normal immunoregulatory T-cell influences. This polyclonal B cell activation is important, because it can cause problems in making a serologic diagnosis of infections that occur subsequent to HIV infection. It may also be associated at times with autoimmune phenomena.
- Variable decreases in functioning of the reticuloendothelial system.

The interaction of the different elements of the immune system in host defense is fairly complicated, beginning with the presentation of antigen by a macrophage to a CD4 T lymphocyte. Subsequent to this a myriad of events occur, including activation of monocytes into macrophages, activation of cytotoxic T cells, induction of natural killer cell activity, induction of antibody production, and regulation of a variety of hematopoietic events, including neutrophil function and eosinophilpoiesis.

For purposes of discussion, one can compare the immune system to a symphony orchestra, the conductor of the immune symphony being the helper-inducer T lymphocyte or the CD4 cell. This is perhaps the most important element in the immune system. This helper-inducer T cell is responsible for recognizing the score, seeing what is going on, distinguishing self from nonself and, after appropriate interpretation of the score, bringing into play the appropriate elements of the immune system, including cytotoxic T cells, natural killer cells, and B cells, often through the elaboration of a variety of growth and differentiation factors or lymphokines.

It is obvious, I think, from this analogy that any event that will target and destroy or impair the helper-inducer T lymphocyte, the conductor of the immune orchestra, takes a major toll on the normal functioning of the immune system. Interestingly, many of these other elements of the immune system are present in the patient with HIV infection; however, they do not function normally because the inductive signals are lost.

Immune Function in HIV Infection

Phenotypic Characteristics of HIV Patients. One of the most straightforward, simplest ways of analyzing any given immune system is through

---

Adapted from the author's Grand Rounds in Medicine presentation at The Mount Sinai Medical Center, Nov. 21, 1989. From the Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD. Address reprint requests to Dr. H. Clifford Lane, Building 10, Room 11B-13, National Institutes of Health, Bethesda, MD 20892.

the phenotypic analysis of T lymphocytes (Fig. 1). In the human immune system, T lymphocytes can be separated into two main components: helper-inducer cells and suppressor-cytotoxic cells. All T lymphocytes have on their surface a T-cell receptor that is associated with the CD3 complex, which mediates signaling for T-cell activation. In addition, the CD4 T lymphocyte has on its surface the CD4 molecule. This protein is thought to be important in facilitating cell-cell interactions, in particular by binding to class II MHC molecules. In patients with HIV-1 infection the CD4 molecule also serves as the receptor for the HIV outer envelope glycoprotein, gp120.

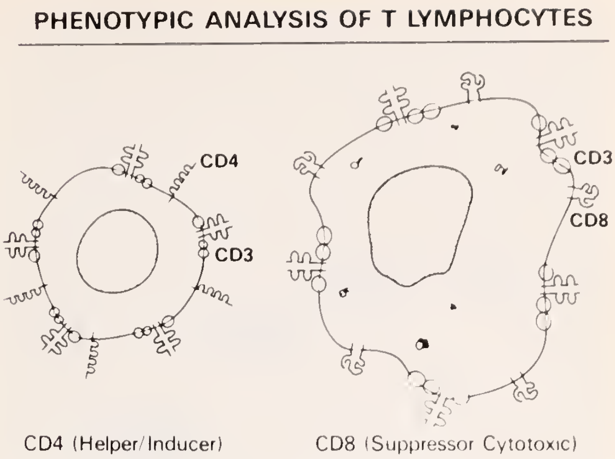

PHENOTYPIC ANALYSIS OF T LYMPHOCYTES
CD4
CD3
CD3
CD8
CD4 (Helper/Inducer)
CD8 (Suppressor Cytotoxic)

Fig. 1. In the human immune system, T lymphocytes can be separated into two main components: helper inducer cells (bearing the CD4 molecule) and suppressor cytotoxic cells (bearing the CD8 molecule).

The CD8 lymphocyte also has a T-cell receptor associated with the CD3 molecular complex and, in contrast to the CD4 cell, has the CD8 molecular complex on its surface. The CD8 molecule is also important in facilitating cell-cell interaction, in this case through interaction with class I MHC molecules.

One can enumerate the number of helper-inducer cells and of suppressor-cytotoxic T cells in an individual through the use of flow cytometry (Fig. 2). These assays begin with samples of peripheral blood mononuclear cells that are then stained with various monoclonal antibodies. If cells are taken from the control individual and stained with an antibody to CD4, a peak appears, corresponding to the presence of CD4 cells in the blood of the control individual. In contrast, cells from an AIDS patient stained for CD4 may produce no peak, indicating very few if any CD4 cells. Staining with an antibody to CD8 results in the appearance of a peak. This peak is present (Fig. 2) both in the control individual and in the patients with AIDS. Thus, phenotypically, the hallmark of the immunologic defect of AIDS is a decrease in CD4 T lymphocytes.

One can use flow cytometry to determine what percentage of mononuclear cells are CD4 positive; if one multiplies that by the total mononuclear cell count (obtained by the white cell count and the differential) one can come up with a CD4 count (Fig. 3). As HIV infection progresses, the CD4 count drops. Patients with Kaposi's sarcoma alone may have relatively high CD4 counts. In contrast, patients with AIDS-defining opportunistic infections almost always have low CD4 counts.

The analysis of CD4 count can be used not only in gauging how far HIV infection has progressed in a patient, but also in the differential diagnosis of pulmonary disease. In fact, in patients with an opportunistic infection as the cause of pneumonia, in particular cytomegalovirus (CMV) pneumonia or *Pneumocystis carinii* pneumonia (PCP), the CD4 count will almost invariably be less than 250. Patients with CMV pneumonia and CMV retinitis generally have CD4 counts under 100. Patients with PCP generally have CD4 counts under 250. In contrast, patients with nonspecific interstitial pneumonitis or pulmonary Kaposi's sarcoma as the cause of a pul-

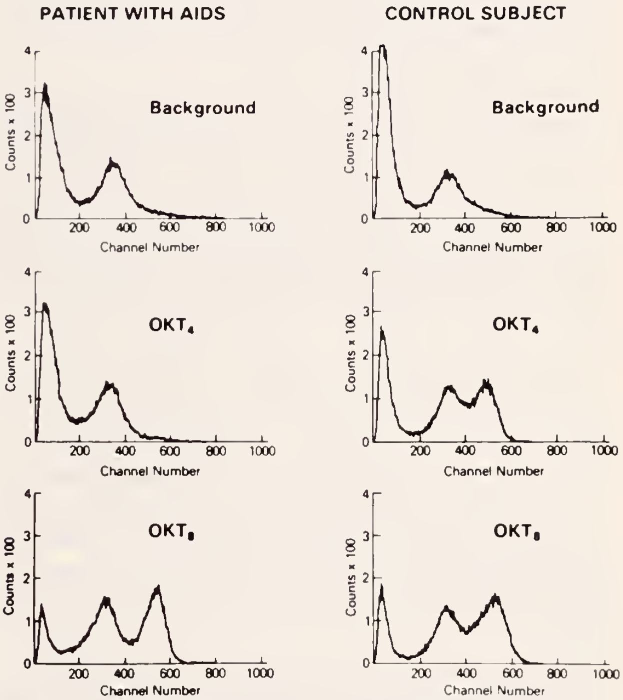


Fig. 2. A series of fluorescent activated cell sorter (FACS) profiles for a patient with AIDS and a control individual. Assays begin with a sample of peripheral blood mononuclear cells that are incubated with a monoclonal antibody. Middle 2 panels: antibody to CD4 has been used. Bottom 2 panels: antibody to CD8 has been used. Cell number is then plotted as a function of fluorescence intensity. Patient with AIDS has a marked decrease in CD4 positive cells.

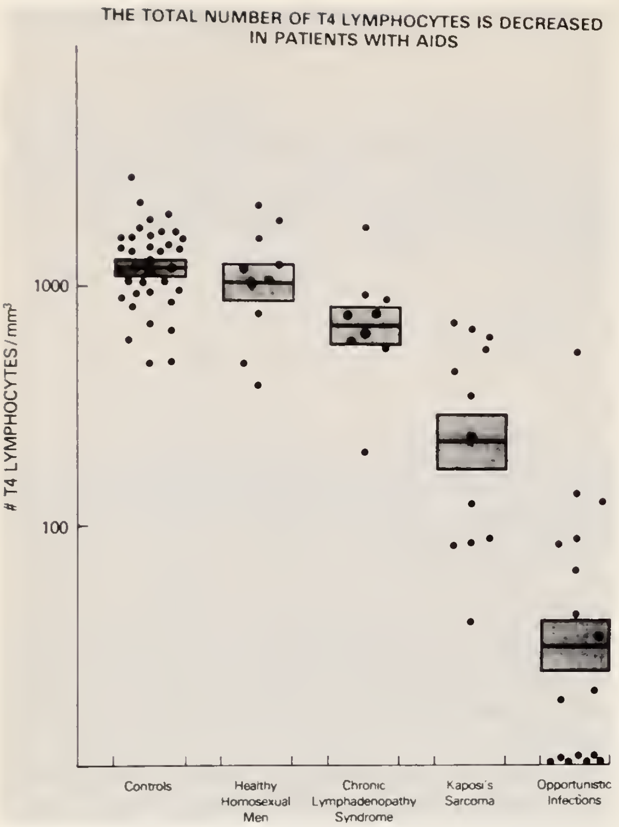


Fig. 3. Total CD4 counts for five groups: HIV seronegative heterosexual controls; HIV seronegative homosexual men; HIV-positive homosexual men with chronic lymphadenopathy; HIV-positive individuals with Kaposi's sarcoma as their sole manifestation of AIDS; and patients with HIV infection and opportunistic infection.

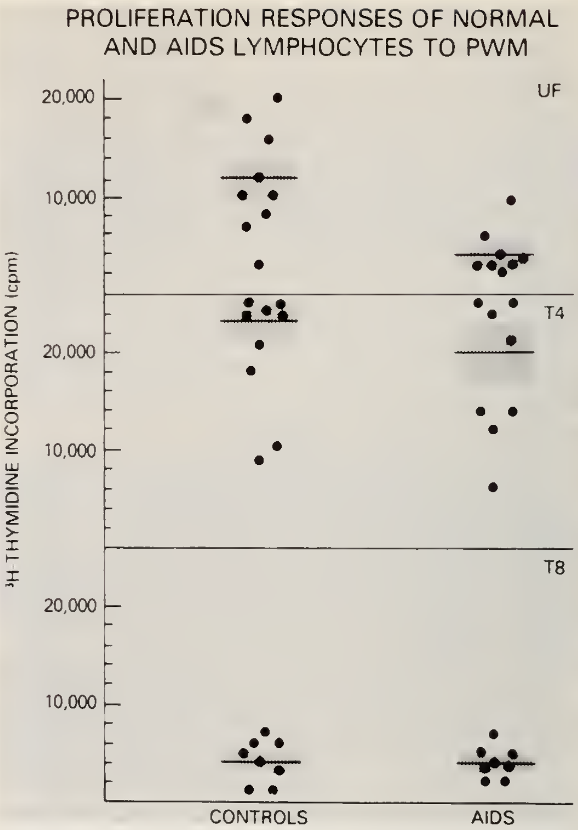


Fig. 4. Proliferative responses of unfractionated, purified CD4 and purified CD8 peripheral blood mononuclear cells from control individuals and patients with AIDS to pokeweed mitogen.

monary infiltrate can have CD4 counts anywhere along the spectrum. Thus, when faced with a patient with HIV infection and pneumonitis, a knowledge of the recent CD4 count may be of help in the differential diagnosis. The CD4 count may help gear thinking as to whether the process is more or less likely to be an opportunistic infection.

In evaluating a series of 55 patients with HIV infection and *P. carinii* pneumonia, we found CD4 counts ranging from 1 to 255 cells/μL (mean 53). The CD4 percentage, another parameter that can be used to monitor patients, ranged from 0% to 25%, mean 6%. Only 2 patients in the series had more than 200 CD4 cells/mm^3, and only 2 patients had a CD4-cell percentage greater than 20%.

It has been the analysis of patients with HIV infection and PCP by these techniques that has led to the current recommendations for the timing of prophylaxis against PCP, namely either HIV infection and a previous bout of PCP or HIV infection, and less than 20% CD4 cells, or less than 200 CD4 cells/mm^3. There appears to be very little need for PCP prophylaxis prior to this point. I know that individual clinicians may or may not use this cutoff. Some prefer to wait a little longer. Patients with 200 CD4 cells who develop *P. carinii* pneumonia appear to have milder cases than patients with 10 or 20 CD4 cells. Thus, these immunologic parameters provide important guidelines in helping to decide when to begin prophylaxis for PCP.

Functional Characteristics of T Cells. The CD4 cell has a variety of functions that can be analyzed precisely in vitro. The CD4 T cell will synthesize DNA and proliferate in response to mitogens, alloantigens, or soluble protein antigens. This ability of the CD4 cell to recognize foreign proteins and proliferate in response to them is a critical element in immunologic surveillance. CD4 T cells also provide help to B lymphocytes to produce immunoglobulin and provide inductive signals for a variety of immunologic functions through the elaboration of a variety of lymphokines.

Lymphocyte proliferation, also referred to as lymphocyte blast transformation, is quantified by measuring DNA synthesis. In this assay, cells are obtained from a patient, then stimulated in the

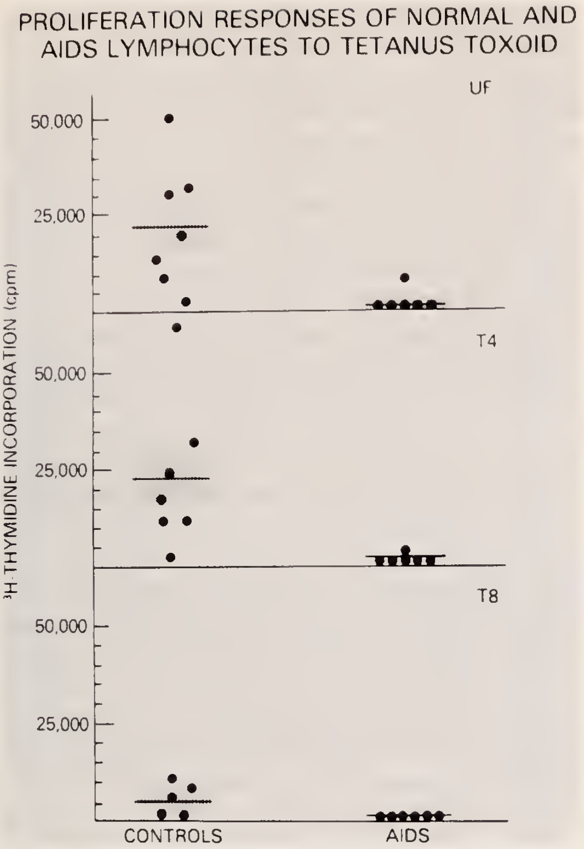


Fig. 5. Proliferative responses of unfractionated, purified CD4 and purified CD8 peripheral blood mononuclear cells from control individuals and patients with AIDS to tetanus toxoid.

test tube either with a nonspecific stimulus, a mitogen such as pokeweed, or a soluble protein antigen, usually a recall antigen such as tetanus toxoid. The cell cultures thus established are allowed to incubate for a set period of time—in the case of pokeweed mitogen, five days, and in the case of soluble antigens, five to six days. At the end of that time, the cultures are labeled with tritiated thymidine, a DNA precursor, and the amount of tritiated thymidine incorporated into DNA is measured. The amount of incorporated radioactivity is a reflection of DNA synthesis, giving an idea of how well the cells have been stimulated.

Both mitogen and antigen activation of T lymphocytes require the participation of accessory cells. In the case of mitogen activation, this is to provide interleukin 1 (IL-1); in the case of antigen activation, in addition to IL-1 production the monocyte functions to process the protein into peptide fragments, which are then reexpressed on the surface of the cell in association with class II MHC molecules.

Analysis of lymphocyte blast transformation of peripheral blood lymphocytes from patients with HIV infection has been helpful in revealing the selective nature of the immune defect in patients with HIV infection (Fig. 4). The proliferative responses of PBMC cells from patients with AIDS to pokeweed mitogen (PWM) are markedly decreased. Further analysis shows that the response is diminished merely because the number of CD4 cells is down, not because of an intrinsic defect in the cells. In fact the responses of purified CD4 cells from AIDS patients to PWM are quite comparable to the responses of CD4 cells from healthy individuals. Although CD8 cells do not respond to pokeweed as well as CD4 cells, the responses for control individuals and AIDS patients are similar. Similar data can be generated for responsiveness to phytohemagglutinin. Thus, the decreased blast transformation to mitogens in AIDS patients is not due to any particular defect in the lymphocytes or the CD4 cells but merely due to the low numbers of CD4 cells. When the decrease in number of cells is taken into account, mitogen responses are normal.

In marked contrast, if one then looks at the ability of these cells to respond to a soluble recall antigen such as tetanus toxoid, the defect persists when correction is made for low number of CD4 cells. Unfractionated cells from patients with HIV infection are markedly defective in responsiveness to the soluble protein antigen tetanus toxoid (Fig. 5). This decrease in responsiveness to tetanus toxoid is not merely a reflection of the decreased CD4 number, because studies of purified CD4 cells reveal the same defect. CD8 cells do not respond to soluble protein antigens and therefore cells from both controls and AIDS patients have low levels of response. Thus, there is a selective defect in the CD4 population of patients with HIV infection, and this selective defect is an inability of these cells to recognize and proliferate in response to soluble protein antigens such as tetanus toxoid.

The response of a CD4 cell to a soluble protein antigen requires that the antigen be taken up by a monocyte or macrophage, processed and reexpressed. To be sure that the defect in antigen responsiveness of CD4 T cells was in fact due to a defect of the T cell and not the monocyte, we studied several pairs of identical twins in which one had AIDS and one did not. We experimented with syngeneic add-backs of T cells from control patients and from AIDS patients to monocytes from the patient or monocytes from the syngeneic controls. Despite adding back graded numbers of monocytes from a healthy control, the tritiated thymidine incorporation of T cells from the patients with AIDS in response to tetanus toxoid

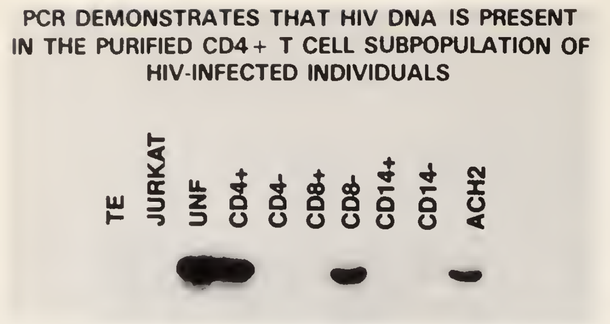

PCR DEMONSTRATES THAT HIV DNA IS PRESENT
IN THE PURIFIED CD4+ T CELL SUBPOPULATION OF
HIV-INFECTED INDIVIDUALS
TE
JURKAT
UNF
CD4+
CD4-
CD8+
CD8-
CD14+
CD14-
ACH2

Fig. 6. PCR analysis of peripheral blood mononuclear cell subsets for HIV-1.

was always totally absent. In contrast, the T cells from the control individuals demonstrated a normal response to tetanus toxoid with the addition of syngeneic monocytes from an AIDS patient. These data indicate that the defect in soluble antigen recognition in patients with HIV infection is at the level of the T cell, not at the level of the monocyte.

In summary, then, the antigen-responsive CD4 T cells are selectively deficient in AIDS patients, and this deficiency appears early in the course of the disease. In fact this defect may be seen even before CD4 numbers begin to drop. Why this occurs, why there is a selective defect in this cell population, is not known. Studies of simian immunodeficiency virus-infected rhesus monkeys have shown similar results. In those studies looking at different cell subsets, the subset of CD4 cells marked by the monoclonal antibody 4B4 (a marker for antigen reactive cells) was selectively decreased.

In trying to understand why the antigen-specific T cell is selectively deficient in patients with AIDS, we have proposed a model in which, in the process of antigen presentation to the antigen-specific CD4 T cell, the T cell may be infected by the macrophage presenting the antigen. Indeed, the macrophage is felt to be a reservoir for HIV infection, and perhaps in the process of antigen presentation one is in fact selectively targeting, infecting, and eliminating the antigen-responsive T cell.

Why the Decrease in CD4 Numbers in HIV-Infected Patients? Many hypotheses have been put forth to explain why the CD4 numbers go down in patients with HIV infection. One thing that has caused people to look for a variety of indirect mechanisms has been the early observation that few cells in the peripheral blood were found to harbor HIV. By in situ hybridization, the precursor frequency of HIV-infected cells was shown to be somewhere in the range of 1:10,000 to 1:100,000. Among the mechanisms that have been postulated to account for the CD4-positive lymphocyte defect in patients with AIDS are productive infection and death of the cells, the accumulation of nonintegrated viral DNA in T lymphocytes (a mechanism of cell death in some animal retroviral systems), immunosuppressive effects of viral proteins (in particular, gp120 blockage of CD4 function), syncytia formation mediated by envelope proteins, and virus-induced alterations in lymphocyte activation.

More recently, analysis of lymphocyte cell subsets from patients with HIV infection by polymerization chain reaction (PCR) has revealed that a substantial portion of CD4-positive T lymphocytes circulating in peripheral blood harbor HIV proviral DNA. In those studies, unfractionated peripheral blood mononuclear cells were separated by flow cytometry into CD4-positive or CD4-negative cells, or into CD8-positive or CD8-negative cells, or into CD14-positive (monocytes) or CD14-negative cells (B and NK cells) by a third sort, this latter sort being done on a T-cell-depleted population. These cell populations were then lysed, and the DNA of the lysed cells analyzed by polymerase chain reaction (PCR) for the presence of HIV proviral DNA.

The unfractionated cells, CD4-positive T cells and CD8-negative cells were all found to have an intense signal for HIV proviral DNA (Fig. 6). Thus, in peripheral blood the predominant cell type harboring HIV appears to be the CD4-positive T lymphocyte. Precursor frequency analysis utilizing serial dilutions of CD4 T cells have shown that the precursor frequency of the CD4-positive T cell that harbors HIV proviral DNA in patients with AIDS can be quite high, at least 1 in 100, and in some instances as high as 1 in 10. In patients with HIV infection the number of CD4-positive cells that are harboring HIV goes up dramatically over time. Fig. 7 depicts changes in PCR signal and CD4 count in a patient with HIV infection followed up for two years. This patient initially was asymptomatic, with a CD4 count of 800 cells per cubic millimeter. A PCR of 10,000 CD4 T cells collected at that time gave a weak signal. Over time, the patient's CD4 count dropped. When his CD4 count was under 200, he developed *P. carinii* pneumonia. A PCR of 10,000 CD4 T cells collected at that time revealed a significantly stronger signal.

Thus, as HIV infection progresses, as the CD4 count is going down, the number of CD4 cells harboring HIV is going up. I think these data strongly support the contention that the immune defect in HIV infection is in fact directly due to HIV infection and destruction of CD4 cells.

Other Immune System Abnormalities in HIV

CD8 T Lymphocytes. The second major T cell of the human immune system is the CD8 T lymphocyte or the T8 cell, the cytotoxic T cell. In a comparison of total CD8 or T8 cell numbers in five groups, including controls, even the seronegative men have an elevation in CD8 count (Fig. 8). Thus, an elevation in CD8 count is part of the normal host immune response to any viral infection. Paradoxically, however, in patients with HIV infection, even though CD8 counts are elevated, CD8 function is down. This is presumably due to a lack of inductive signals from the CD4 cells.

CD8 cells have two main functions in host defense. They suppress antibody production and they mediate T-cell-specific cytotoxicity. Both these parameters appear to be defective in patients with HIV infection, not because of an intrinsic defect of the CD8 cells, but because of the loss of the inductive signals provided by the CD4 T cells. CD8 cells from AIDS patients will suppress antibody production normally if CD4 cells from a healthy donor are present in culture.

Defects in cytotoxic cell function are seen in both virus-specific T-cell-mediated cytotoxicity and natural killer cell cytotoxicity. The measurement of cell-mediated cytotoxicity is a straightforward immunologic assay that measures how well one cell population induces the lysis of another. In this assay, one takes a population of effector cells, often peripheral blood mononuclear cells, and incubates them, either immediately or following stimulation, with the target cells. The target cell is labelled by an incubation with radioactive chromium (^{51}Cr). The ^{51}Cr gets into the cell and if the cell is killed following incubation with the effector cells, chromium is released into the culture supernatant. One can determine how many of the target cells have been killed by how much chromium is released into the culture supernatant. These data are generally expressed as percent lysis or percent ^{51}Cr release.

Virus-specific cytotoxicity is a T-lymphocyte-mediated function. It is generally due to the interaction of a CD8 cell with a virus-infected target cell. This interaction initially occurs between the T-cell receptor of the CD8 cell and viral antigens expressed on the surface of the virus-infected cell in association with class I MHC molecule. In patients with HIV infection, decreases are seen in both natural killer cell activity and T-cell-specific cytotoxicity. Levels of NK activity are variably depressed. In some patients with HIV infections and very low CD4 counts, natural killer cell activity may be essentially absent. Similarly, CMV-specific cytotoxicity is usually absent in patients with HIV infection. This is a recurrent theme in studying the immune systems of patients with HIV infections, namely that spe-

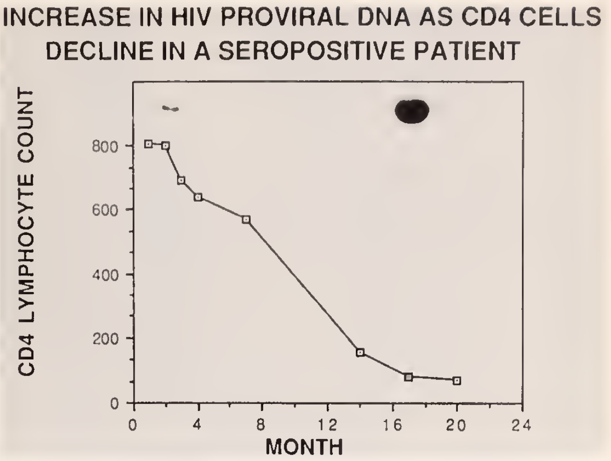


Fig. 7. Changes in CD4 count and in the intensity of the HIV-1 PCR signal in CD4 T lymphocytes from a patient with HIV infection followed over a period of two years.

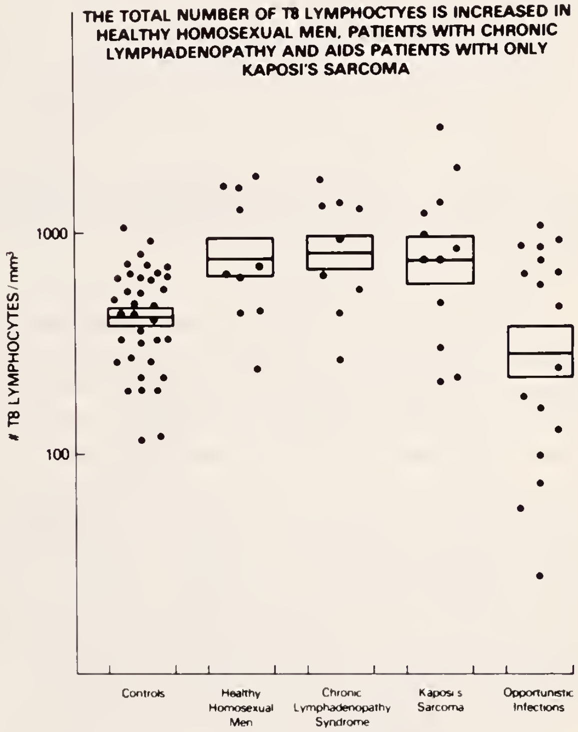


Fig. 8. Total CD8 or T8 cell numbers for a group of heterosexual controls, a group of HIV-negative homosexual men, a group of HIV-positive homosexual men with chronic lymphadenopathy syndrome, patients with AIDS manifested only by Kaposi's sarcoma, and patients with AIDS manifested by an opportunistic infection.

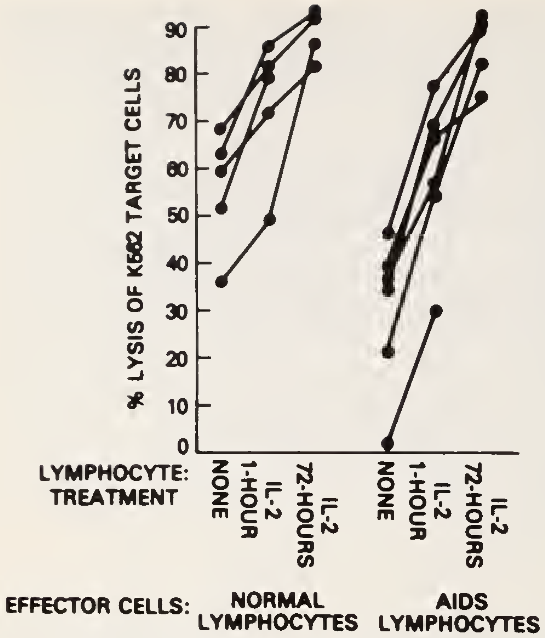


Fig. 9. Defective NK activity of peripheral blood mononuclear cells from patients with AIDS can be reversed in vitro by the addition of interleukin 2. Data are expressed as percent lysis of target cells as a function of duration of exposure to IL-2 for a group of control individuals on the left and a group of patients with AIDS on the right.

cific immune responses are the most severely depressed. Although not functional, the CMV-specific cytotoxic T cell is present in the peripheral blood of HIV-infected patients. It is not able to function because of the lack of inductive signals from the CD4 T cell. These defects in cytotoxicity can be reversed in vitro by the addition of lymphokines, in particular interleukin 2 (Fig. 9), a T cell derived protein produced by CD4 cells used to induce other cells to proliferate or differentiate.

These in vitro observations of the ability of IL-2 to boost levels of NK activity provided the preclinical data base for using interleukin-2 as a biologic response modifier in patients with HIV infection. Clinical trials are currently underway to see whether the immune systems of patients being treated with zidovudine (AZT) can be boosted by interleukin 2 treatment. Because of the importance of cytotoxic T cells in host defense against viral infection and against neoplasm, it is felt that if one can boost cytotoxicity in a sustained way in patients with HIV infection, one may have a way of ameliorating some of the complications of immunodeficiency.

B Cell Activation. A variety of abnormalities of B-cell activation are present in patients with AIDS. In vivo, one sees elevated levels of serum IgG and IgA (Fig. 10) with normal levels of IgM. In another seeming paradox, even though the serum levels of IgG and IgA are elevated in these patients, there is a decrease in their ability to make specific antibodies following immunization. In other words, the B cells of patients with HIV infection are polyclonally activated, as in patients with certain autoimmune diseases. As a result the system loses its ability to respond to new antigenic stimuli. This makes the serologic diagnosis of disorders such as CMV or toxoplasmosis unreliable.

In vitro a variety of abnormalities of B cell activation can be seen, among them elevated spontaneous blast transformation. The B lymphocytes are already activated, they are dividing, and will incorporate tritiated thymidine without exogenous stimulation. In addition, these B cells have enhanced responsiveness to growth factors and an enhanced propensity to spontaneously secrete immunoglobulin.

Even though these patients exhibit polyclonal hypergammaglobulinemia, they have a decreased ability to respond to a new antigenic stimulus. This has been documented in a series of studies utilizing a variety of antigens, including the potent primary protein antigen keyhole limpet hemocyanin (KLH).

This defect in specific antibody production in patients with AIDS is generally present in the later stages of HIV infection. Earlier in the course of infection, antibody responsiveness may be quite good. Thus, if one is going to consider immunizing patients, say with pneumococcal polysaccharide, the time to immunize is early in the course of HIV infection. Later on, the HIV-infected patient may have no serologic response. Similarly, when one is trying to make a diagnosis of reactivation toxoplasmosis or reactivation CMV, serologic markers are often unreliable, since both these infections generally occur late in the course of HIV infection.

The reticuloendothelial system or the macrophage-monocyte system is variably defective in patients with HIV infection. Decreases occur in Fc receptor function as measured by defects in the clearance of antibody-coated red cells, chemotaxis of monocytes, and cytotoxicity of monocytes. Immunologically the reticuloendothelial system appears to be intact in that antigen presentation and interleukin-1 production are normal.

Summary

In summary, patients with HIV infection have a variety of immunologic defects. The hallmark defect is a decrease in the number and function of helper-inducer or CD4 T lymphocytes. A decrease in CD4 count to less than 200 is associated with the development of opportunistic infections. The earliest functional defect is in soluble antigen recognition. Although there is an increase in the number of suppressor cytotoxic or CD8 T lymphocytes, there is a decrease in their function, presumably due to a lack of inductive signals from the CD4 T cells. Decreases in natural killer cell function occur, as does polyclonal B cell activation, which makes serologic diagnosis of opportunistic infections somewhat unreliable. Finally, there are also variable decreases in the function of the reticuloendothelial system.

Questions and Answers

Lloyd Mayer: Thank you for a heroic presentation. With the NIH experience and rather large experience with AZT, have you looked at the soluble antigen defect in patients after AZT treatment? If there is a decrease in viral load, then theoretically they may improve their ability to recognize soluble antigen.

Lane: In monitoring patients on a variety of experimental therapies, including AZT, we have never seen any treatment cause a restoration of soluble antigen recognition and proliferation, with the exception of transient improvement following bone marrow transplantation. So none of the chemotherapeutic agents we currently use have been able to restore that particular defect.

Mayer: Do you think that goes against your theory that viral load plays an important role in this defect?

Lane: No. I think what happens is that the defect occurs so early that we're unable to shift the viral load back far enough. So I think that AZT, while it may decrease the viral burden, is not capable of pushing the burden back outside of a critical area. AZT slows progression rather than significantly restoring immune function.

Question: Have you studied the relationship between HIV infection determined by PCR and by seropositivity?

Lane: We have not done that in a population of homosexual men with low CD4 counts. We have run both tests in a large cohort of seronegative homosexual men with normal CD4 counts in the context of a phase one study of a candidate HIV vaccine, a gp160 envelope protein. In recruiting individuals for that study we were looking at polymerase chain reaction as well as Western blot and P24 antigen. We have not found, in screening individuals for that study, anyone who was polymerase chain-reaction positive without some other marker of HIV infection. We have not observed the polymerase chain reaction being positive long before other markers turn positive.

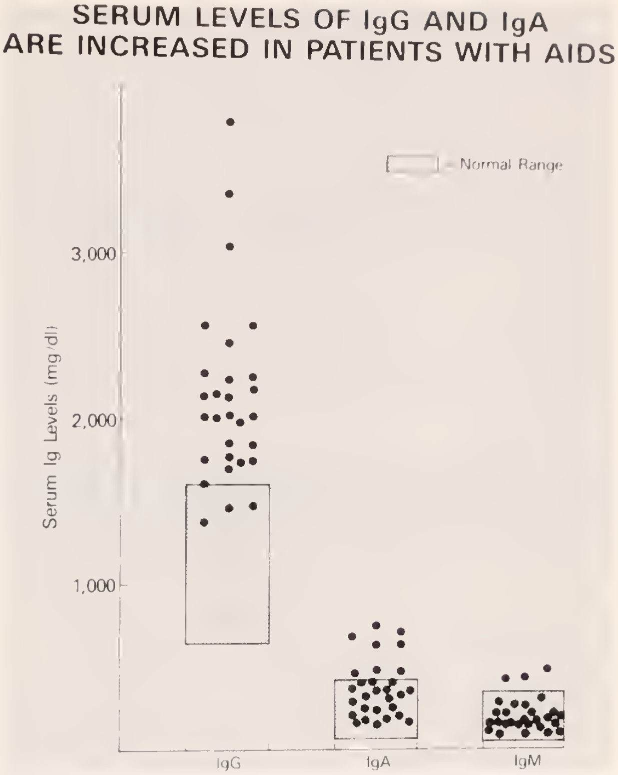


Fig. 10. Serum IgG, IgA and IgM levels in a cohort of patients with HIV infection. Control values are represented by the shaded areas.

Question: In the study in which you immunized individuals to KLH, you had three homosexual controls who did about a third as well as the controls. Were these homosexuals HIV positive?

Lane: That's actually a fairly old study that was done before the days of HIV. What we had in that group were two people who were HIV positive and one who was HIV negative, and in fact it was the one who was HIV negative that brought the mean response of that group up.

Question: In terms of the influence of HIV infection on the response to immunization, there seems to be an across-the-board suggestion that everyone be immunized early to minimize problems with influenza and pneumococcae. What are your recommendations with respect to vaccination of the HIV-infected individuals?

Lane: The Centers for Disease Control recommend immunization against flu and pneumococcus. In our clinic we generally do not use influenza immunization. We have not had problems with severe cases of influenza in patients with HIV infection. The part of the immune system that deals with influenza appears to be functioning quite normally. If the patient wants influenza immunization, however, we do provide it to them. The more serious problem is, as you suggest, postinfluenza pneumonias. We do immunize our patients with pneumococcal polysaccharide.

Question: I want to get back to the possible monocyte defects. On the studies that were done with identical twins, did you do the reverse study, taking the monocytes from the affected individual and trying to stimulate a tetanus response that was normal?

Lane: If you take monocytes from an HIV-infected individual and add them back to monocyte-depleted T cells from a normal individual, what you'll see, doing titrations of monocytes from both control and HIV-infected individuals, is about a 50% shift in the dose-response curve, the monocytes from the patient showing a slightly lower response. We have studied three identical twin pairs in this manner. Thus, you can get maximum antigen presentation by monocytes from HIV-infected persons; however, you have to add a few more cells.

Question: Are monocytes from AIDS patients heavily burdened with HIV?

Lane: Circulating monocytes do not appear to contain HIV. The only monocytes or macrophages I know of that have been shown to contain HIV have been fixed tissue cells.

Grand Rounds

Primary Care for Patients with HIV

Joseph R. Masci, M.D.

I do not intend to present here an all-encompassing algorithm for the care of patients with HIV infection or AIDS. I am offering, instead, some perspective on where practical management stands in 1991 from the standpoint of a clinician who sees HIV-infected patients. I also provide a glimpse of our program at Elmhurst and how and why it has evolved the way it has.

Epidemiologic Data

Patterns of AIDS Infection. In the all-too-familiar charting of the AIDS epidemic in the United States, the slope of the curve is gradually declining; for the most recent year, an apparent drop in cases always appears on the curve (Fig. 1) (1). This, unfortunately, reflects reporting delays rather than a true drop. As of April 1991, the total number of cases of AIDS in the United States is approximately 170,000 (2), the number of deaths approximately 100,000. Worldwide statistics on AIDS are less precise because of reporting problems in many countries, but the number is in the range of 300,000 to 500,000 reported cases of AIDS, and perhaps two or three times that many not reported.

The epidemic curve in New York City is comparable to the national curve, although the slope is somewhat flatter (Fig. 2) (3). The total number of cases in New York by the end of January 1991 was approximately 30,000 (4); the number of deaths was almost 20,000. The apparent fall-off in 1990 is probably not real but most likely reflects reporting delays. In the early days of the epidemic, in 1981, about 37% of all new cases of AIDS were reported annually from New York; in 1991 that percentage had dropped to only about 10%. Manhattan has the largest number of cases, followed by Brooklyn, Bronx, Queens, and Staten Island.

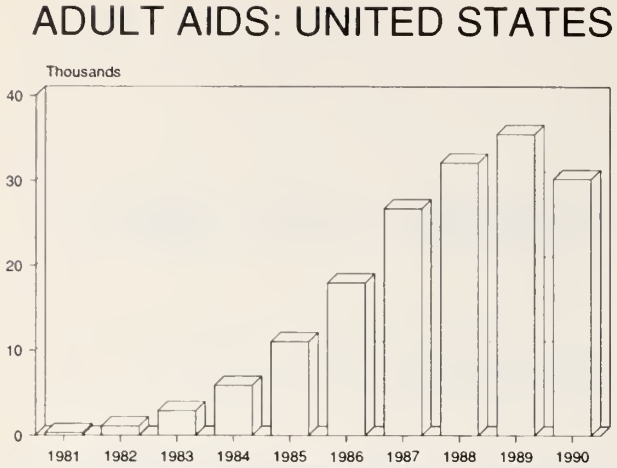
In the United States, those who are infected with the human immunodeficiency virus, type 1 (HIV) are still predominantly men. Epidemiologists classify countries into three patterns for HIV infection. Pattern one, characteristic of the United States, Europe, South America, and Australia, is a preponderance of males, particularly male homosexuals and intravenous drug users. Pattern two, primarily countries in Central Africa and the Caribbean, is characterized by a more equal sex distribution in which male homosexuality and intravenous drug abuse do not appear to be important factors. Pattern three countries are those in which AIDS transmission is minimal.

In the breakdown by risk group nationally (Table 1), a handful of cases among health-care workers have been reported (included in risk group “other”). The heterosexual contact category has received much attention, but in reality the heterosexual component of reported AIDS has not been climbing fast. It has gradually increased but has not skyrocketed, despite fears and some predictions.

Women are an important component in patterns of AIDS infection. Some epidemiologists believe that pattern one countries, those in which AIDS victims are now predominantly homosexual men and intravenous drug users, are going to gradually turn into pattern two countries with time, through heterosexual transmission to women. Following the percentage of cases among heterosexual women gives an indication of how rapidly the United States may be transforming into a pattern two country. Still, over the course of the entire epidemic the majority of women in-

---

Adapted from the author’s Grand Rounds in Medicine presentation at the Mount Sinai Medical Center on April 9, 1991. From the Department of Medicine, Elmhurst Hospital Center, Elmhurst, NY. Address reprint requests to the author, Associate Director of Medicine, Mount Sinai Services, Elmhurst Hospital Center, 79-01 Broadway, Elmhurst, NY 11373.



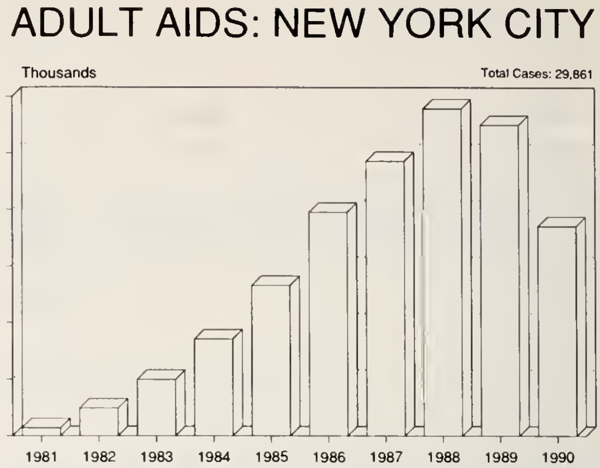


Fig. 1. Left Adult AIDS cases reported to Centers for Disease Control, 1981–1990. *Adapted from* ref. 1. **Fig. 2.** Right Adult AIDS cases reported to the New York City Department of Health, 1981–1990. *Adapted from* ref. 3.

fected have been intravenous drug users. In the period from pre-1985 through 1990, however, heterosexually acquired cases among women with AIDS gradually increased (5).

The age distribution among infected women is comparable to that among men: the peak is in women of childbearing age, between the ages of 15 and 45. And that, of course, spells pediatric AIDS, which is a second implication of the female AIDS epidemic in this country. The vast majority of children with AIDS and with HIV infection are infected in utero or at or around the time of delivery by an infected mother. That is, there is a two-dimensional aspect and tragedy to the AIDS epidemic among women.

Patterns of HIV Infection. AIDS statistics are not the whole story. AIDS has often been likened to an iceberg. The visible part is the actual reported cases, for which AIDS is still a fairly narrowly defined syndrome. A patient has to have an opportunistic infection or malignancy or a specific AIDS-related syndrome. Subclinical cases and asymptomatic patients are not reported at this point, although the case definition is anticipated to expand in early 1992. The entire iceberg in the United States, that is, the number of patients actually infected with HIV, is in the range of 700,000 to 3 million, varying by statistical model. The majority of patients who are HIV infected are still asymptomatic. Unfortunately, most patients who are diagnosed as HIV infected are diagnosed at the time that they develop AIDS. So despite ten years of the epidemic and the health care system response, HIV infection is still diagnosed quite late in most patients.

TABLE 1

Risk Groups of Reported Adult AIDS Patients: United States through February 1991

Risk group	No. (%)
Male homosexual/bisexual	97,687 (59%)
Intravenous drug user	36,155 (22%)
Both	10,916 (7%)
Heterosexual contact	8,907 (5%)
Transfusion	3,787 (2%)
Hemophilia/coagulopathy	1,434 (1%)
Other* or undetermined†	6,014 (4%)
Total	164,900

* Other, includes cases among health-care workers.

† Undetermined, cases in which interviewing is incomplete.

The larger issue of HIV seroprevalence in the United States can be viewed from various perspectives (Table 2) (6–18).

One large cohort study (6) in San Francisco has shown that, after the discovery of HIV, the etiologic agent of AIDS, and the elucidation of patterns of transmission and risk behavior, enough behavioral modification took place among the male homosexual community in San Francisco to significantly alter transmission rates; seroprevalence has been falling. The number of AIDS cases among male homosexuals is still enormous, but that risk group's proportion of AIDS cases is falling. In contrast to the situation with homosexual men, no significant change in transmission rates among intravenous drug users seems to have taken place.

Heterosexual partners of, for example, intravenous drug users also have a high prevalence of HIV infection. Heterosexual transmission rates, however, are not consistent from study to study. In one study of wives of male hemophiliacs (19), only 9% became infected over a prolonged period of follow-up. Yet other data (20) suggest a much higher rate of spread by this route. These reported differences in rates have not been completely explained.

Although prevalence in large general population groups is low, among military applicants the seroprevalence is tenfold higher than among marriage license applicants, and fourfold or fivefold higher than among blood donors—an alarming fact. Military applicants are young, they tend to be lower on the socioeconomic scale in this country, minorities are overrepresented, and they come disproportionately from inner-city areas. The military data may be a sign of the future of the epidemic more revealing than the blood-donor or marriage-license studies.

Specific patient populations yield prevalence rates which are intermediate between high-risk groups and the general population.

In the Sentinel Hospital Surveillance study (21), HIV infection was found among hospitalized patients in every state studied, although prevalence rates varied widely in a geographic pattern similar to that of reported cases of AIDS. This is data that may be important in determining risks to health care workers. If emergency room patients selected randomly in Baltimore have a one-in-twenty chance of being HIV infected, then the risk to health care workers is presumably more substantial there than in other parts of the country.

Data at Elmhurst. Data from our own clinic at Elmhurst between 1988 and the end of 1989, about 18 months, shows how the AIDS iceberg is reflected in practical terms in New York City. At Elmhurst the proportion of asymptomatic HIV-positive patients is increasing. On the one hand, we are pleased to be identifying HIV-positive patients prior to the onset of AIDS. On the other hand, the curve is alarming; it has continued upward in a smooth slope through 1990 (Fig. 3).

Impact on the Specialty of Infectious Diseases. AIDS is not the only negative force on the shape of medicine today. Another alarming trend is that in this country interest in internal medicine residencies is declining. The number of post-graduate year 1 residents is gracefully and elegantly sliding each year (Fig. 4) (22). More alarming is the even more rapid decline in the number of new fellows in infectious disease (Fig. 5) (22).

These two curves, the AIDS epidemic and enrollment in medicine and infectious disease training programs, although they are going in opposite directions, are on something of a collision course, I think. AIDS, so far, has been largely an epidemic of the subspecialities—infectious diseases, hematology, oncology, dermatology, neurology, pulmonology—and that may have to change. The numbers may become so overwhelming that the disease may have to move more into the medical mainstream, just so that we can provide care for all of these patients.

TABLE 2

Prevalence of HIV Infection: United States (Selected Series)

Group (ref. no.)	%
High Risk	
Male homosexuals, San Francisco (6)	48.5
Drug users, New York City (7)	55–60
Factor VIII concentrate recipients, pre-1985 (8)	78
Heterosexual partners of IV drug users with AIDS (9)	48
Low Risk	
Blood donors (10)	0.038
Military applicants (11)	0.15
Marriage license applicants, Illinois (12)	0.011
Other	
Childbearing women	
Massachusetts (13)	0.21*
New York (14, 15)	2.0†, 2.7‡
STD clinic, Baltimore (16)	5.2
Hospital inpatients, Washington, D.C. (17)	3.7
Emergency room patients, Baltimore (18)	5.2

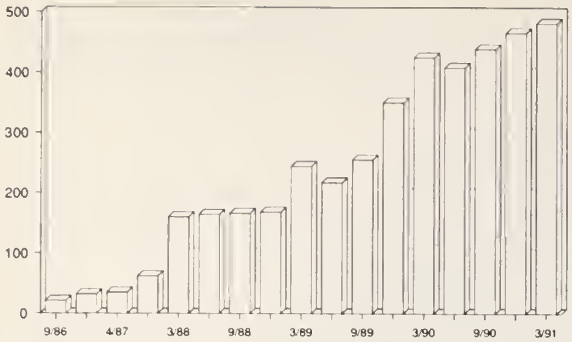
* Range: 0.09% in suburban and rural hospitals to 0.8% in inner city hospitals.

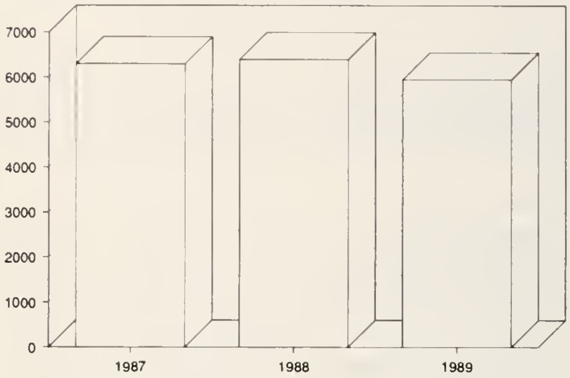
† Municipal hospital.

‡ Voluntary hospital.

Adapted from Masci JR. Primary and ambulatory care of the HIV infected adult. St. Louis: Mosby-Yearbook Co., 1992.

The impact of the AIDS epidemic on infectious disease practice in New York City has been staggering. Five years ago, our clinic met every other week; we had two to three interesting parasitology cases; we were done by 9:30 in the morning, and that was the end of it. Now, we're greeted by a tidal wave of AIDS and HIV patients five days a week, morning and afternoon. Five years ago no one at our hospital was working exclusively on AIDS, now 43 employees at Elmhurst in all categories of jobs are working exclusively on AIDS. What was initially perhaps the most consultative of subspecialities—infectious diseases—has been transformed for many of its practitioners into a paradigm of a primary care specialty.





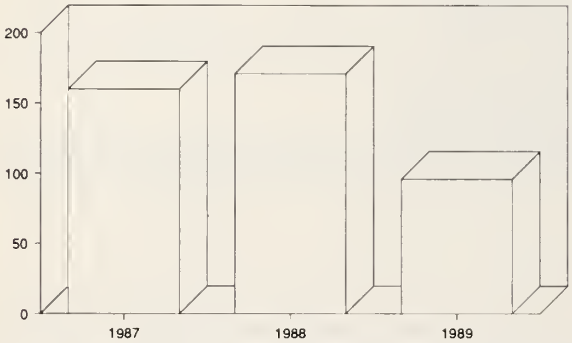


Fig. 3. Growth in number of visits to AIDS Clinic at Elmhurst Hospital Center, Sept. 1986–March 1991. Fig. 4. Decline in number of postgraduate year 1 medical residents, 1987–1989. *Adapted from* ref. 22. Fig. 5. Decline in number of infectious diseases fellows, 1987–1989. *Adapted from* ref. 22.

We are taking care of patients who require screening; interpreting a vast number of clinical syndromes; making appropriate referrals to subspecialists; counseling and providing nursing and social service care.

Practical Management

What the Internist Must Know about HIV Infection. The three major elements of AIDS care that any internist practicing in New York has to have mastered are (a) the indications for and meaning of HIV testing; (b) clinical and immunologic staging of the disease; and (c) the interpretation of common HIV-related clinical findings.

The indications for HIV testing are

- history of male homosexuality
- history of intravenous drug abuse
- history of blood transfusion, especially pre-1985
- sexual partner is person at risk
- history of a sexually transmitted disease
- history of prostitute exposure
- origin in or travel to a country classified as pattern 2
- patient believes he or she might be infected

Much is sometimes made of the difficulty physicians encounter in taking sexual histories from patients. The material routinely mailed to physicians from the Centers for Disease Control and the state and city departments of health, for instance, often includes elaborate instructions on how to take such histories. Indeed, surveys have shown that medical students and house officers, as well as attending physicians, are often particularly deficient in that aspect of history taking (23). That deficiency has been pointed out as an obstacle in the response of the medical profession to the AIDS epidemic. In other words, if a physician cannot feel comfortable asking a patient if they have a history of homosexuality or other risk behavior, then the physician really cannot provide appropriate screening for HIV infection to patients in general.

That makes a kind of sense, however simplistic, but I believe the argument has fallacies. Even when a sexual history is taken, one study (24) has shown that simply asking patients about risk factors may only identify about 60% of those who are infected, although eliciting an accurate sexual history should identify virtually all infected by the sexual route, according to the AIDS statistics.

The second fallacy is that one actually has to confront the issue of homosexuality or intravenous drug use or heterosexual partners who are at risk in order to advise testing. One might, in fact, simply say to the patient: these are the risk factors; if you are at risk, let's test you. In other words, effective screening for HIV infection may not require the elaborate sexual history-taking which for many physicians may represent an obstacle. Not that I'm against getting a complete history, but there may not be the luxury anymore of waiting for behavior patterns among physicians to change.

Methods of Testing for HIV Infection. Several techniques are used to test for HIV infection, either by antibody detection, or antigen detection. The enzyme-linked immunosorbin assay, or ELISA, is the commonly used screening antibody test that is performed on blood. ELISA is the initial screening test. Western blot is the confirmatory test. Other methods may be used to detect viral antigens. These include culture, p24 antigen, polymerase chain reaction. These latter techniques, however, are still basically research tools. They are not well standardized, and they are not easily available to most practitioners.

The routine testing procedure is today what it has been since 1985. Blood is tested first by ELISA. If it is positive, the test is repeated. If the second test is also positive, the specimen is tested again by the Western blot technique. If that test is positive, the laboratory reports the result as positive. Occasionally, blood is reported as indeterminate by Western blot, and in those cases patients should be retested. Patients who are at high risk and test indeterminate are usually proven to be positive; those who are at low risk who test indeterminate are often negative.

HIV antibody testing is performed in a variety of different places. We send all of our specimens for routine testing to the New York City Department of Health Laboratory. It is an efficient process. We get results back within five to seven days.

In the summer of 1989, when studies began to indicate that early treatment of HIV-infected patients is beneficial, the entire philosophy of testing changed at Elmhurst, in New York City, and nationwide. Prior to 1989 a legitimate argument could be made that testing was of no clinical value. It not only raised issues of confidentiality but conferred no therapeutic benefit on the patient because no effective treatment existed for any stage of HIV infection. That is not the case anymore, and this change has prompted massive screening efforts. At Elmhurst today any patient admitted to the medical service with an HIV-suspicious diagnosis, for example someone with cellulitis in the arm who we suspect might be an intravenous drug abuser, is approached and offered voluntary HIV testing.

The philosophy has changed from almost never testing to urging testing in every remotely appropriate setting. The ELISA and the Western blot are both highly sensitive and specific. Concerns that were raised earlier about false positives have largely been addressed, and there are newer testing strategies on the way incorporating other more refined tests that almost completely eliminate issues of imprecision of testing.

Staging of HIV Infection. After establishing the diagnosis of HIV infection, the next step is to undertake clinical and immunologic staging. We have learned a great deal about the natural history of HIV infection. Infection is chronic and permanent. An asymptomatic phase lasts for many years and is the lengthiest phase of the illness in most patients. Infection is transmissible throughout all stages of the disease. A progressive and irreversible depletion of CD4 lymphocytes, the so-called helper-inducer cells, occurs relatively late in HIV infection.

The clinical manifestations of HIV disease predate AIDS itself by many years in many individuals (24). Not all of the manifestations of HIV infection reflect immune dysfunction.

In large studies aided by mathematical modeling the median time before developing AIDS appears to be in the range of 8 to 11 years, perhaps somewhat shorter when infection is by blood transfusion, and somewhat longer when infection occurs in homosexual males. In other words, this is a chronic disease, not unlike many of the other diseases we deal with in treating adults. Unfortunately, it is a chronic disease affecting a young age group.

Markers of Disease Progression. What does the physician notice when someone is progressing through HIV infection (24–29)? The clinical markers of disease progression (29A) are

- Oral candidiasis (25)
- Oral hairy leukoplakia (26)
- Weight loss (24)
- Fever (24)
- Severe Herpes zoster (27)
- Advanced age (28, 29)

Why is clinical and immunologic staging of HIV infection necessary? For one reason, because the data on therapy have been derived in a way that forces physicians to stage. What was established first is that AZT, the only currently licensed antiviral agent, was effective in patients who had AIDS or AIDS-related complex (30). It was subsequently shown (31) to be effective in patients who had CD4 counts below 500, regardless of symptoms. It has not yet been shown to be effective in other settings. Thus the clinical application of AZT therapy currently requires immunologic staging, as does the timing of preventive therapy, particularly against *Pneumocystis carinii,* and, perhaps most important of all, the interpretation of clinical events.

To illustrate this last point: when I take residents' report on the medical service, we often get into a discussion of an HIV-infected patient whose initial symptom may represent a life-threatening disease or may be trivial. As I have reminded the residents many times, the immunologic staging of the patient is absolutely critical

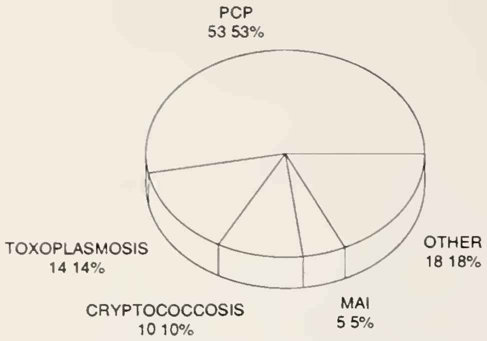


Fig. 6. AIDS-defining diagnoses in first 500 patients at Elmhurst Hospital Center; numbers in percent.

in the interpretation of complaints such as headache, new fever, new cough, new diarrhea, new weight loss. One of the things that makes rational care for HIV patients possible is that the immune system deterioration is predictable and clearly related to clinical events. Patients with a CD4 lymphocyte count over 200/mm^3, for example, almost never develop *Pneumocystis* pneumonia or any other opportunistic respiratory infection (32). This means that when a patient with a CD4 lymphocyte count of 500 develops pneumonia it is likely to represent a routine, not an opportunistic, infection. Fortunately, the vague concept that because HIV-infected patients are immunocompromised and that therefore any new sign or symptom can mean disaster is overly simplistic and pessimistic. We can take a much more refined approach than that.

Antiviral Therapy. If the CD4 count is less than 500/mm^3, the National Institutes of Health currently recommends institution of antiviral therapy (33). Now, despite this recommendation, there are many things that we do not know about the use of AZT in asymptomatic patients who have less than 500 CD4 cells. We do know that in a population of patients who were studied in the ACTG trials, it appeared to be beneficial, leading to lower mortality as well as less frequent and less severe opportunistic infections. What we do not know is whether it is much more effective in some subsets of patients and much less effective in others, so that there's a balancing out. There has even been the assertion that because of the possibility of the virus becoming resistant to AZT with time, initiating therapy with AZT or any other antiretroviral drug early in the disease may ultimately prove counterproductive. But for now we are limited by what has been demonstrated in prospective clinical trials. And that does justify the institution of AZT therapy at a CD4 count of 500 or below.

Below 200 total CD4 cells or below 20% of the total lymphocyte count, prophylaxis against *Pneumocystis* is recommended, and at any lymphocyte count, certain immunizations, which I list below, are recommended. That is the extent of our basic interventions. There is nothing else that we do routinely for all HIV-infected patients.

The negative side of AZT therapy is its toxicity. Only rarely do any patients using it for more than a year or so not develop significant anemia, although recent data suggest that the earlier the therapy begins and the lower the dose, the longer it can be used without prohibitive toxicity.

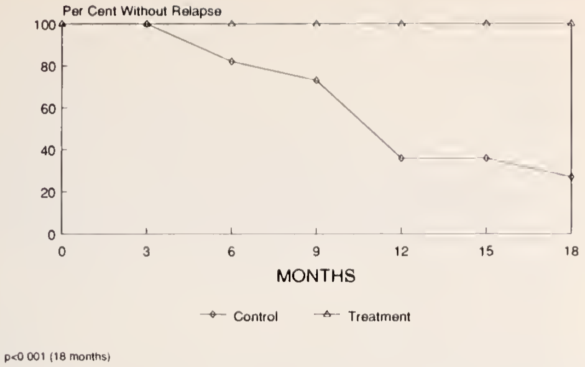
Two other antiviral compounds with activity against HIV are dideoxyinosine (DDI) (34) (available) and dideoxycytidine (DDC) (35), which is in the final clinical-trials stages and expected to be available soon. Although it is not clear that either agent is superior to AZT in efficacy or toxicity, they may find a place either as replacement therapy or as follow-up therapy when patients become overly toxic on AZT or in combined regimens. Other compounds which have yielded encouraging results in early studies include soluble CD4 (36) and interferon alpha (37).

So AZT is our primary antiviral agent at the moment. Recent actuarial data presented at the international AIDS conference in 1990 suggested that AZT alone across the entire population of HIV-infected patients so far treated with it may add 1½ years to life expectancy (38). Another important parameter is an improvement in the Karnofsky score, a behavioral index by which we grade the individual's capacity to function physically, which tends to improve during initial therapy with AZT.

The major side effects of the antiviral drugs are, for AZT, anemia and leukopenia; for DDI, peripheral neuropathy and pancreatitis; and for DDC, peripheral neuropathy.

***Pneumocystis* prophylaxis.** Among the first 500 AIDS patients diagnosed at Elmhurst, the mortality from *Pneumocystis carinii* pneumonia was 35% (Fig. 6).

Pneumocystis prophylaxis has been one of the major advances in the management of HIV-infected patients, and the regimens currently recommended by the CDC (39) are trimethoprim sulfa, which is Bactrim or Septra, given twice daily (40), or inhaled pentamidine given every four weeks by nebulizer. Trimethoprim sulfa, in our retrospective data at Elmhurst, is 100% effective in preventing *Pneumocystis* infection. Inhaled pentamidine is slightly less effective (41).



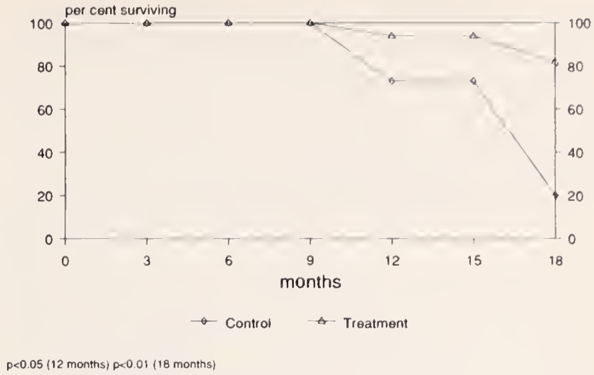


Fig. 7. **Left** Follow-up of patients receiving trimethoprim-sulfamethoxazole as secondary prophylaxis for *Pneumocystis carinii* pneumonia, Elmhurst Hospital Center. *Adapted from* ref. 40. **Fig. 8. Right** Mortality of patients receiving treatment with trimethoprim-sulfamethoxazole as secondary prophylaxis for *Pneumocystis carinii* pneumonia, Elmhurst Hospital Center. *Adapted from* ref. 40.

Other alternatives, dapsone and fansidar, have not been studied quite as extensively but may prove useful.

At Elmhurst we collected retrospective data on *Pneumocystis* relapse among patients who had already had *Pneumocystis* once. These patients were maintained on Septra therapy, double-strength twice a day; controls were retrospective; results are shown in Figs. 7 and 8.

The combination of AZT and *Pneumocystis* prophylaxis using pentamidine has also been shown to lead to enhanced survival (42).

Immunizations. Vaccination against *Hemophilus* (43) and pneumococcal infection (44) as well as influenza (43), hepatitis B when indicated (45), and the childhood illnesses are recommended for patients at various stages of disease. These guidelines (43–45) should be reviewed, especially before the administration of live virus vaccines.

To summarize, these are the routine measures in the current approach to the generic therapeutic and preventive management of the HIV-infected patient:

- Staging
  Lymphocyte subset analysis
  Screening
- VDRL, PPD, PAP smear
- Immunization
- *Pneumocystis* prophylaxis (if indicated)
- Antiretroviral therapy (if indicated)
- Counseling, contact assessment

Note that at Elmhurst we screen all patients for syphilis and tuberculosis, regardless of immunologic stage. All women have initial screening gynecologic examinations, including PAP smears.

Most physicians are familiar with the common AIDS-related opportunistic infections and malignancies. However, many nonspecific problems are encountered in HIV-infected patients.

Diagnostic and Therapeutic Algorithms. Recently, diagnostic and therapeutic algorithms for HIV-infected patients have been published, which is gratifying to the clinician. There have been recommendations on how to evaluate various HIV-related syndromes.

The predictive value of oral thrush and esophageal symptoms for *Candida* esophagitis have been studied (46) so we can determine which patient with esophageal symptoms needs to undergo endoscopy and when barium radiography will suffice.

More precise information on the etiology of seizures (47) and on causes of abdominal pain (48) have also been gathered.

As other advances are occurring in AIDS—more is being learned about the virus, and clinical trials of antiviral therapy and prophylaxis are progressing—parallel clinical reports on practical management issues are also starting to appear, and that is important. Physicians not particularly focused on the literature on AIDS must often think it absolutely bewildering in volume; in fact in Medline recently I found a request for “the acquired immunodeficiency syndrome” yielded 24,000 citations (and that number is rising rapidly). Buried in those 24,000 citations are some clinically useful algorithms. Although much of the literature is clinically useful, the primary-care approach is beginning to appear as a new dimension in the literature.

The Experience at Elmhurst. At Elmhurst, our AIDS inpatient census parallels the New York City epidemic curve. We have never been inundated the way some other municipal hospitals have, because the prevalence of HIV infection in Queens is relatively low. At our clinic the number of patients has multiplied many times since September 1986, when we saw roughly 10 or 20 patients; we are currently following 1200 patients, a huge staff commitment (Fig. 9).

Some of these patients come to the clinic because they have been hospitalized at Elmhurst, but many have not. More and more referrals come from the community, from the anonymous testing sites run by the Department of Health. Our own data show that the patients from the Department of Health, who are generally asymptomatic and have sought testing because they are at risk, are often quite immunodeficient; 40% had CD4 counts below 200 and had to immediately begin *Pneumocystis* prophylaxis.

In addition to medical care we offer extensive support services. We have been criticized and often questioned about why these services are not also available for persons with congestive heart failure, for example, or diabetes. I'm not sure I have a good answer to that. I think it may be because AIDS evolved in front of our eyes, and because it is an affliction of young people whose lives are devastated completely and suddenly.

Summary

AIDS will soon emerge as one of the top five causes of death in the United States. By 1995 it is going to be roughly equivalent to stroke in this regard: about 200,000 deaths annually. By that year between 400,000 and 500,000 AIDS cases will have been reported in this country.

Right now our health care system, which is so sophisticated but which can be so inadequate, is effectively addressing AIDS in some areas of the country but is not effectively addressing the HIV epidemic. In order to identify HIV infection early, we all have to be involved. As internists we have to routinely offer screening. We have to make it easily accessible to our patients. There should be no more thought of ignoring the question of HIV infection than of ignoring smoking. There is no other answer to this major public health dilemma, and despite all the AIDS centers that are developing and the funding for in-patient and out-patient facilities, I think they are all going to be overwhelmed eventually, particularly in New York City, if we do not develop a more broad-based approach to the problem.

Questions and Answers

Question: Could you say a little about what you do about a partner of someone you find to be HIV positive?

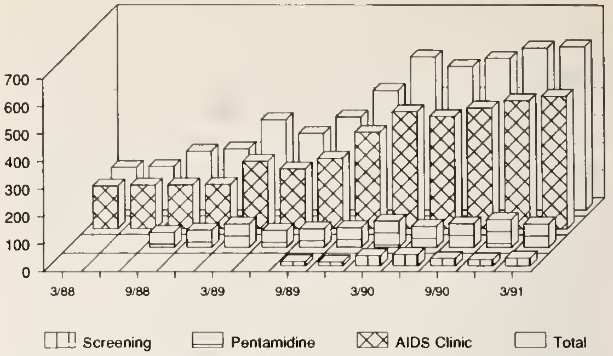


Fig. 9. Distribution of outpatients by HIV clinic category, Elmhurst Hospital Center.

Masci: There are some legal constraints. You are obligated to ask a patient whom you've tested to inform their partner. If you reasonably believe that they'll do so, your legal responsibility ends there. If you do not believe they'll notify their partner, you are legally able to take the step of informing the partner. We have instead suggested the compromise of having the Department of Health notify the partner that someone they've been in contact with over the years is HIV infected and they should be tested. Obviously, that is more appropriate for couples that aren't married. With married couples we have in the end always been able to get the spouse to inform the partner.

Question: Are there any specific attributes of acute retroviral illness?

Masci: The description of the retroviral illness is broad and nonspecific. Many times over the years we've had health care workers who got needle sticks and a week or two later developed something that had features of the retroviral illness. None have proven to be infected with HIV. What the retroviral illness specifically includes is myalgias, sore throat, sometimes a fine macular rash, lymphadenopathy, sometimes ulcerations in the mouth. If you see a patient with all of those symptoms, days to weeks after a potential exposure, I suppose you should have a suspicion that the exposure resulted in infection. But similar syndromes are common, particularly in the wintertime, and may be difficult to interpret.

Question: What you present really has important implications as far as teaching is concerned—specialists, housestaff. Yet look at programs throughout the city. Many AIDS clinical centers have segregated their AIDS patients, both on the ambulatory service and the in-patient services. My question, therefore, is, do you have a segregated in-patient service, and if you do not and you've scattered your AIDS patients as they come to the house, how do you involve a housestaff, and what recommendations on AIDS patients ought to be made for teaching institutions?

Masci: A good point. We do not have a segregated in-patient unit. We have a segregated out-patient operation, though, and what we're trying to do is involve the primary care clinic at Elmhurst in at least the early care of HIV-infected patients, and also to accept their residents as on elective, rotating through our clinic. We haven't met with tremendous success in that. It is a problem of perception, I think. There is also a perception that it has been the infectious group that has grown, that has been given the funding, so that it is sometimes hard to convince people that it is important for residents to see AIDS patients. What I've found, though, is that the residents themselves are more and more seeing the importance of it. We have residents requesting to take our elective at this point, and that is different from a couple of years ago. I think residents who trained in New York are beginning to realize that they are going to be expected to know about AIDS, they are going to be expected to know how to handle it. But we do not have a good solution or even a long-term plan at this point for how to make that educational effort grow.

Question: I have two questions about testing. One concerns the patient who wants to get tested but cannot easily get an anonymous test through the physician's office, through the physician's own lab. We must realize that many patients do not want their blood to go to the Department of Health when there is the possibility of a positive.

The second is the patient who doesn't want to get tested, and there I'm thinking of the house officer who gets a needle stick from a patient. How do you handle this?

Masci: On the first issue: the Department of Health runs an anonymous testing program that is impressive. It is run through the AIDS Hotline, there are anonymous testing sites all over the city. That is the solution to the patient for whom anonymity is an important concern—to offer the option of not testing them through your office but giving them the phone number of the anonymous testing site where they are tested and informed anonymously. Then it is up to them to come back to you with the results if they decide to.

As for the house officer who gets a needle stick, there are a lot of emotional issues in HIV testing that aren't necessarily apparent immediately. Finding out that you're HIV positive is so devastating that I think we have to respect an individual's desire to not find out, as long as they are not placing other people at high risk, as long as we do not believe that the risk was great, as is the case in most needle sticks. It is hard to force the issue, and in fact legally you cannot. All you can do is give your best advice, urge testing, and then leave it up to the individual that maybe if they are not tested immediately, they'll subsequently see the light and decide that it is the best thing for them. But fortunately, the risk to health care workers is small.

References

1. Centers for Disease Control. HIV/AIDS Surveillance. December 1990.
2. Centers for Disease Control. HIV/AIDS Surveillance. April 1991.
3. New York City Department of Health. AIDS Surveillance Update. April 30, 1991.
4. Centers for Disease Control. HIV/AIDS Surveillance. February 1991.
5. Centers for Disease Control. HIV in women—United States. MMWR 1990; 39:845–846.
6. Winkelstein W, Jr., Samuel M, Padian NS, et al. The San Francisco Men's Health Study III. Reduction in human immunodeficiency virus transmission among homosexual/bisexual men, 1982–86. Am J Public Health 1987; 77:685–689.
7. Des Jarlais DC, Friedman SR, Novick DM, et al. HIV-1 infection among intravenous drug users in Manhattan, New York City from 1977 through 1987. JAMA 1989; 261:1008–1012.
8. Lederman MM, Ratnoff OD, Evatt BL, McDougal JS. Acquisition of antibody to lymphadenopathy-associated virus in patients with classic hemophilia (factor VIII deficiency). Ann Intern Med 1985; 102:753–757.
9. Harris CA, et al. HTLV-III/LAV infection and AIDS in heterosexual partners of AIDS patients. Paper presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, Minn., September 29–October 2, 1985.
10. Schorr JB, Berkowitz A, Cumming PD, Katz AG, Sandler SG. Prevalence of HTLV-III antibody in American blood donors. N Engl J Med 1985; 313:384–385.
11. Burke DS, Brundage JF, Herbold JR, et al. Human immunodeficiency virus infections among civilian applicants for United States military service, October 1985 to March 1986: demographic factors associated with seropositivity. N Engl J Med 1987; 317:131–136.
12. Turnock BJ, Kelly CJ. Mandatory premarital testing for human immunodeficiency virus: the Illinois experience. JAMA 1989; 261:3415–3418.
13. Hoff R, Berardi VP, Wieblen BJ, Mahoney-Trout L, Mitchell ML, Grady GF. Seroprevalence of human immunodeficiency virus among childbearing women: estimation by testing samples of blood of newborns. N Engl J Med 1988; 318:525–530.
14. Landesman S, et al. Serosurvey of human immunodeficiency virus infection in parturients: implications for human immunodeficiency testing programs of pregnant women. JAMA 1987; 258:2701–2703.
15. Sperling RS, Sacks HS, Mayer L, Joyner M, Berkowitz RL. Umbilical cord blood serosurvey for human immunodeficiency virus in parturient women in a voluntary hospital in New York City. Obstet Gynecol 1989; 73:179–181.
16. Quinn TC, Glasser D, Cannon RO, Matuszak DL, Dunning RW, Kline RL. Human immunodeficiency virus

infection among patients attending clinics for sexually transmitted diseases. N Engl J Med 1988; 318:197–203.

17. Gordin FM, Gibert C, Hawley HP, Willoughby A. Prevalence of human immunodeficiency virus and hepatitis B virus in unselected hospital admissions: implications for mandatory testing and universal precautions. J Infect Dis 1990; 161:14–17.
18. Kelen GD, Fritz S, Qaqish B, et al. Unrecognized human immunodeficiency virus infection in emergency department patients. N Engl J Med 1988; 318:1645–1650.
19. Kreiss JK, Kitchen LW, Prince HE, Kasper CK, Essex M. Antibody to human T-lymphotropic virus type III in wives of hemophiliacs: evidence for heterosexual transmission. Ann Intern Med 1985; 102:623–626.
20. Harris CA, et al. HTLV-III/LAV infection and AIDS in heterosexual partners of AIDS patients. Paper presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, Minn., September 29–October 2, 1985.
21. St. Louis ME, Rauch KJ, Petersen LR, Anderson JE, Schable CA, Dondero TJ. Seroprevalence rates of human immunodeficiency virus infection at sentinel hospitals in the United States. N Engl J Med 1990; 323:213–218.
22. Rowley BD, Baldwin DC, McGuire MB, Etzel SI, O'Leary CJ. Graduate medical education in the United States. JAMA 1990; 264:822–832.
23. Merrill JM, Laux LF, Thornby JI. Why doctors have difficulty with sex histories. South Med J 1990; 83:613–617.
24. Lifson AR, Rutherford GW, Jaffee HW. The natural history of human immunodeficiency virus infection. J Infect Dis 1988; 158:1360–1367.
25. Klein RS, Harris CA, Small CB, Moll B, Lesser M, Friedland GH. Oral candidiasis in high-risk patients as the initial manifestation of the acquired immunodeficiency syndrome. N Engl J Med 1984; 311:354–358.
26. Greenspan D, Greenspan JS, Hearst NG, et al. Relation of oral hairy leukoplakia to infection with the human immunodeficiency virus and the risk of developing AIDS. J Infect Dis 1987; 155:475–481.
27. Melbye M, Grossman RJ, Goedert JJ, Eyster ME, Biggar RJ. Risk of AIDS after herpes zoster. Lancet 1987; 1:728–731.
28. Eyster ME, Gail MH, Ballard JO, Al-Mondhiry H, Goedert JJ. Natural history of human immunodeficiency virus infection in hemophiliacs: effects of T-cell subsets, platelet counts and age. Ann Intern Med 1987; 107:1–6.
29. Goedert JJ, Kessler CM, Aledort LM, et al. A prospective study of human immunodeficiency virus type 1 infection and the development of AIDS in subjects with hemophilia. N Engl J Med 1989; 321:1141–1148.

29A. Masci JR. Primary and ambulatory care of the HIV-infected adult. St. Louis, MO: Mosby-Yearbook Co., 1992.

30. Fischl MA, Richman DD, Grieco MH, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. N Engl J Med 1987; 317:185–191.
31. Volberding PA, Lagakos SW, Koch MA, et al. Zidovudine in asymptomatic human immunodeficiency virus infection: a controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. N Engl J Med 1990; 322:941–949.
32. Crowe SM, Carlin JB, Stewart KI, Lucas R, Hoy JF. Predictive value of CD4 lymphocyte numbers for the development of opportunistic infections and malignancies in HIV-infected persons. J Acq Immune Def Synd 1991; 4:770–776.
33. National Institutes of Health. Recommendations for zidovudine: early infection. JAMA 1990; 263:1606–1607.
34. Lambert JS, Seidlin M, Reichman RC, et al. 2',3'-dideoxyinosine (DDI) in patients with the acquired immunodeficiency syndrome or AIDS-related complex. N Engl J Med 1990; 322:1333–1340.
35. Broder S, Yarchoan R. Dideoxycytidine: current clinical experience and future prospects: a summary. Am J Med 1990; 88:5B–31S–33S.
36. Schooley RT, Merigan TC, Gaut P, et al. Recombinant soluble CD4 therapy in patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex: a phase I–II escalating dosage trial. Ann Intern Med 1990; 112:247–253.
37. Lane HC, Davey V, Kovacs JA, et al. Interferon-alpha in patients with asymptomatic human immunodeficiency (HIV) infection: a randomized, placebo-controlled trial. Ann Intern Med 1990; 112:805–811.
38. Peters BS, Beck EJ, Coleman DG, Moss F, Harris JRW, Pinching AJ. The role of zidovudine in increased survival in AIDS patients. Paper presented at Sixth International Conference on AIDS, San Francisco, Calif, June 21, 1990.
39. Centers for Disease Control. Guidelines for prophylaxis against Pneumocystis carinii pneumonia for persons infected with human immunodeficiency virus. MMWR 1989; 38(S-5):1–9.
40. Pierone G, Masci J, Nicholas P. Trimethoprim-sulfamethoxazole for secondary prophylaxis of Pneumocystis carinii in AIDS. Paper presented at the Fifth International Conference on AIDS, Montreal, June 6, 1989.
41. Golden JA, Chernoff D, Hollander H, Feigal D, Conte JE. Prevention of Pneumocystis carinii pneumonia by inhaled pentamidine. Lancet 1989; 1:654–657.
42. Beck EJ, Peters BS, Coleman DG, Harris JRW, Pinching AJ. Changing morbidity and mortality patterns in British AIDS patients. Paper presented at Sixth International Conference on AIDS, San Francisco, Calif, June 21, 1990.
43. Centers for Disease Control. Recommendations of the Advisory Committee on Immunization Practices (ACIP): immunization of children with human immunodeficiency virus—supplentary ACIP statement. MMWR 1988; 37:181–183.
44. Centers for Disease Control. Recommendations of the Advisory Committee on Immunization Practices: pneumococcal polysaccharide vaccine. JAMA 1989; 261:1265–1267.
45. Hibberd PL, Rubin RH. Approach to immunization in the immunosuppressed host. Infect Dis Clin North Am 1990; 4:123–142.
46. Connolly GM, Forbes A, Gleeson JA, Gazzard BG. Investigation of upper gastrointestinal symptoms in patients with AIDS. AIDS 1989; 3:453–456.
47. Holtzman DM, Kaku DA, So YT. New-onset seizures associated with human immunodeficiency virus infection: causation and clinical features in 100 cases. Am J Med 1989; 87:173–177.
48. Barone JE, Gingold BS, Arvanitis ML, Nealon TF, Jr. Abdominal pain in patients with acquired immune deficiency syndrome. Ann Surg 1986; 204:619–623.

Grand Rounds

Pulmonary Complications of HIV Infection

Mark J. Rosen, M.D.

In 1981, a report was published on a group of patients with *Pneumocystis carinii* pneumonia, who had no previously recognized cause of immunosuppression (1). These individuals were among the first cases of the acquired immunodeficiency syndrome, the most important global public health problem of the twentieth century. Now in its second decade, the AIDS epidemic continues to accelerate. As of January 1992, 202,921 cases of AIDS were reported in the United States, and 133,233 have died; 45,506 new cases were reported in 1991 alone (2).

Since the first reports, pulmonary diseases have been among the most frequent AIDS-defining diagnoses and account for a high proportion of morbidity and mortality. However, new trends in the epidemiology of HIV infection, the increasing use of antiviral therapies, and prophylactic strategies against *Pneumocystis carinii* are likely to change the spectrum of pulmonary complications in HIV-infected individuals (Table).

Pneumocystis carinii Pneumonia

In 1983, the National Institutes of Health convened a workshop on the pulmonary complications of AIDS at which investigators from centers across the United States shared their collected experience in the diagnosis and outcomes of pulmonary disorders in patients classified as having AIDS by criteria set forth by the Centers for Disease Control (3). *Pneumocystis carinii* pneumonia (PCP) occurred in 373 of 441 patients (85%), either alone or in association with a second pathogen, and PCP is widely perceived as the most common pulmonary disease in patients with HIV infection.

Diagnosis. Occasionally the initial symptoms of the disease are those of an acute illness with rapid deterioration over a few days. More often, it progresses insidiously, with gradually increasing cough and dyspnea over weeks or even months. The chest roentgenogram typically reveals bilateral alveolar and interstitial infiltrates, but unusual manifestations may occur, including nodular densities, cystic lesions, pneumothorax, lobar consolidation, or normal x-rays (4). Furthermore, the typical x-ray pattern for PCP is not specific, occurring in a variety of other infections and neoplastic disorders.

The diagnosis of PCP depends on identification of the organism from specimens derived from the lung. The least invasive diagnostic modality is the induction of sputum with 3% saline delivered by ultrasonic nebulization. When performed by experienced staff, *P. carinii* can be identified by modified Giemsa, methenamine silver, or immunofluorescent staining in 50%–80% of cases (5–7). Occasionally, other pathogens, particularly *M. tuberculosis*, may also be found using appropriate staining and culture techniques.

If sputum induction is not diagnostic, the next step should be fiberoptic bronchoscopy and bronchoalveolar lavage. In the diagnosis of PCP, some have recommended that transbronchial biopsy be omitted initially, to be performed as part of a second bronchoscopy if the lavage is nondiagnostic (8). However, biopsy is occasionally diagnostic when lavage is negative in PCP, and is the least invasive means of diagnosing other pulmo-

---

Adapted from the author's Grand Rounds in Medicine Presentation at the Mount Sinai Medical Center on May 7, 1991. From the Division of Pulmonary and Critical Care Medicine, Beth Israel Medical Center, New York. Address correspondence to the author, chief, Division of Pulmonary and Critical Care Medicine, Associate Professor of Medicine, Mount Sinai Medical School, Beth Israel Medical Center, First Avenue at 16th Street, New York, NY 10003.

TABLE

Pulmonary Complications of AIDS

Infections
Protozoa
Pneumocystis carinii
Strongyloides stercoralis
Toxoplasma gondii
Bacteria
Streptococcus pneumoniae
Hemophilus influenzae
Staphylococcus aureus
Moraxella catarrhalis
Mycobacterium tuberculosis
Mycobacterium avium intracellulare
Fungi
Cryptococcus neoformans
Histoplasma capsulatum
Coccidioides immitis
Aspergillus fumigatus
Viruses
Cytomegalovirus
Adenovirus
Herpes simplex
Other Complications
Lymphocytic interstitial pneumonitis
Nonspecific interstitial pneumonitis
Bronchiolitis obliterans organizing pneumonia
Primary pulmonary hypertension
Malignancies
Kaposi's sarcoma
Non-Hodgkin's lymphoma

nary conditions that require histologic interpretation. Therefore, the advisability of performing transbronchial biopsy routinely during the evaluation of a patient suspected of having PCP is controversial. It is contraindicated in the presence of bleeding disorders, and the high risk of pneumothorax generally precludes biopsy in patients undergoing mechanical ventilation.

In patients with respiratory failure who have an endotracheal tube in place, bronchoalveolar lavage can be accomplished without the use of the fiberoptic bronchoscope. A diagnosis of PCP was established in 19 of 20 (95%) intubated patients by infusing six 10-mL aliquots of sterile saline into the endotracheal tube, removing the fluid by standard suctioning techniques, and performing appropriate microbiologic analyses on the fluid sediment (9). Similar high diagnostic yields can be achieved by placing a 12 Fr nasogastric tube blindly through the endotracheal tube until slight resistance is met, and obtaining bronchoalveolar lavage fluid exactly as if a bronchoscope were used. When lavage yields no diagnosis in an intubated patient, open-lung biopsy should be considered to establish the diagnosis.

Adjunctive testing is also useful in the diagnosis of PCP. Most patients with PCP have an elevation of serum lactic dehydrogenase (LDH); a normal level is compelling evidence against this infection (10, 11). However, an elevated LDH level may occur in other pulmonary diseases. Similarly, the gallium-67 lung scan is almost always positive in cases of PCP, but is also nonspecific (4). This test should be reserved for patients with normal results on chest roentgenogram in whom an opportunistic infection is suspected because of symptoms. If there is abnormal gallium uptake in the lungs, further diagnostic studies aimed at demonstrating an opportunistic infection are appropriate, whereas if the scan results are negative, observation alone is appropriate for most patients (8).

Knowledge of the degree of immunosuppression as estimated by CD4 lymphocyte counts may also be of value in formulating diagnostic approach. Masur et al., in a retrospective analysis of 100 patients with suspected pulmonary complications of HIV infection, found that 80 of 85 episodes of PCP and other opportunistic infections were associated with a CD4 count below 200/mm^3 in the preceding two months (12). In contrast, patients with pulmonary Kaposi's sarcoma or nonspecific interstitial pneumonitis had CD4 counts greater than 200/mm^3. The authors calculated that if the CD4 count was greater than 300/mm^3 within the prior two months, the likelihood that pneumonia was due to PCP was less than 10%, but if the count was less than 100/mm^3, the likelihood was 67.2%.

Similarly, Phair et al. found that PCP is unusual in individuals with CD4 counts greater than 200/mm^3 and when it occurs, patients commonly have thrush and antecedent fever (13). Therefore, HIV-infected individuals known to have CD4 lymphocyte counts over 400/mm^3 within the past two months who have respiratory symptoms but no chronic manifestations of HIV infection (fatigue, fever, thrush, unintentional weight loss) or x-ray abnormalities are unlikely to have an opportunistic infection.

Unfortunately, the high prevalence of PCP in individuals with respiratory complaints who are known to have low CD4 lymphocyte counts and pulmonary symptoms has led to an increase in the use of empiric therapy without a thorough diagnostic evaluation. A specific etiologic diagnosis should be obtained whenever possible, because antimicrobial treatment of *P. carinii* is frequently associated with significant toxicity, and many patients with "classic" presentations in fact do not have this infection. If a patient is treated empirically and does not have PCP, effective therapy may be delayed for many days, with potentially dire consequences.

Treatment. The two most commonly used therapies for PCP are trimethoprim-sulfamethoxazole (TMP-SMX), 15–20 mg/kg/day of TMP, and 75–100 mg/kg/day of SMX, or pentamidine isoethionate, 4 mg/kg/day, administered intravenously. The optimal duration of treatment is unknown, but a consensus has emerged that 14 to 21 days is necessary. Both regimens have approximately equal efficacy, and carry substantial risk of toxicity (14, 15).

Recent studies suggest that alternative therapies may be effective and less toxic in mild-to-moderate episodes, defined as an arterial PaO_2 breathing room air greater than 70 mm Hg (16). These regimens include oral TMP-SMX, trimethoprim-dapsone (17), clindamycin-primaquine (18), and aerosolized pentamidine (19–21). Intravenous medications are not necessary, and these alternatives appear promising for outpatient treatment, but definitive studies still need to be performed before wide application of the alternatives.

Corticosteroids. Recent research indicates that corticosteroids, administered together with antimicrobial therapy, can lead to more rapid improvement in symptoms and gas exchange and increases survival in patients with *P. carinii* pneumonia.

A report by a National Institutes of Health–University of California panel reviewed five randomized, placebo-controlled trials and concluded that adjunctive corticosteroid therapy given at the start of anti-*Pneumocystis* treatment reduces the likelihood of respiratory failure, deterioration of oxygenation, and death in patients with moderate to severe pneumonia (22). Patients likely to benefit had an arterial oxygen tension less than 70 mm Hg or an arterial-alveolar oxygen gradient above 35 mm Hg. No benefits were proven for patients with less severe abnormalities in gas exchange at the start of therapy, or for patients in whom corticosteroids were administered more than 72 hours after antimicrobial treatment was begun.

The optimal dosage and duration of corticosteroid treatment is unknown. The largest controlled study that showed benefit used oral prednisone in the following regimen: on days 1 through 5, 40 mg given twice daily; on days 6 through 10, 40 mg given daily; on days 11 through 21, 20 mg given daily (23). Adverse reactions occurred infrequently, and life-threatening infections were seen in only one study, in which corticosteroid therapy was administered in higher doses to patients who had established respiratory failure (24).

The mechanism by which corticosteroids benefit patients with *P. carinii* pneumonia is also unknown. Montaner and associates noted deterioration in gas exchange during the first few days of anti-*Pneumocystis* therapy in a group of patients who were not given corticosteroids (25). This effect may have been caused by an accelerated inflammatory response to killed organisms. Corticosteroids attenuate this worsening of gas exchange, perhaps by reducing inflammation.

Prophylaxis. Strategies to prevent the development of PCP have been studied in recent years, with the expectation that prevention will decrease both the incidence of this common infection and its attendant mortality (26). The two compounds studied most extensively have been TMP-SMX given orally, and aerosolized pentamidine.

The first randomized controlled trial of TMP-SMX prophylaxis in HIV-infected patients compared with placebo a dose of trimethoprim, 160 mg, plus 800 mg sulfamethoxazole (1 double-strength tablet) given twice daily in combination with leucovorin, 5 mg once daily (27). The patients who received prophylaxis had a lower incidence of PCP and longer survival, but adverse reactions to TMP-SMX occurred in one half of the patients treated. Lower doses of TMP-SMX appear to be effective and better tolerated. A recent retrospective analysis confirmed the efficacy of a lower dose regimen (1 double strength tablet every other day), where only one case of PCP was diagnosed in 521 patient-months of observation (28). Lower-dose regimens hold out the possibilities of an inexpensive, effective preventive measure with a lower incidence of adverse reactions. Furthermore, TMP-SMX may also help to prevent the emergence of other HIV-associated infections, including bacterial pneumonias and toxoplasmosis. The safety and efficacy of this approach is now being investigated in prospective controlled trials.

Pentamidine administered by aerosol has the potential advantages of high drug levels in the lung with minimal systemic absorption and toxicity (29). It is effective prophylaxis against PCP in patients with HIV infection at a dose of 300 mg given every four weeks (30). The most common adverse reactions to the treatments are mild cough and wheezing, reversible with bronchodilators.

PCP may still occur despite aerosolized pentamidine administration, but the rate of prophylaxis failure is not precisely known. When it occurs, it is associated with unusual clinical and diagnostic features. Normal regional differences in ventilation make upper lobe distribution of the drug less concentrated than in the lower lung zones, accounting for a higher rate of atypical, predominantly upper lobe involvement (31, 32). These patients are also more likely to have cystic lung lesions and pneumothoraces, and the diagnostic yields of gallium scans and bronchoalveolar lavage are reduced (32). Finally, many cases of extrapulmonary *P. carinii* have been reported among patients on aerosolized pentamidine prophylaxis, presumably because the drug is distributed only in the lung (33).

A large controlled trial comparing the safety and efficacy of TMP-SMX with pentamidine for prophylaxis of PCP is in progress. Alternative regimens of unproven efficacy and safety are also in use and under investigation, including dapsone, dapsone-trimethoprim, dapsone-pyrimethamine, sulfadoxine-pyrimethamine, and clindamycin-primaquine (26).

Bacterial Pneumonias

Most recent studies of the pulmonary complications of AIDS name PCP as the most common, largely because only patients with CDC-defined AIDS are considered. Pneumonia caused by common bacterial pathogens is not diagnostic of AIDS but appears to occur with greater frequency in HIV-infected individuals than in their seronegative counterparts (34, 35), probably because of abnormalities in humoral immunity that occur as a consequence of defects in cellular immune function (36).

Recent evidence suggests that bacterial pneumonias occur more commonly than PCP in patients with HIV infection (37). This may be due to a declining incidence in PCP among individuals taking prophylactic medications, and in part to changes in the epidemiology of AIDS, with increasing numbers of intravenous drug users developing symptomatic HIV infection (2).

Bacterial pneumonias usually produce typical initial symptoms: high fever, shaking chills, cough productive of purulent sputum, and localized areas of consolidation on chest x-ray. However, many patients have diffuse pulmonary infiltrates that are similar to those commonly seen in PCP (37). Patients are often bacteremic and critically ill (38). *S. pneumoniae* and *H. influenzae* are the most common pathogens, and the diagnostic and therapeutic approaches to these patients are similar to those for HIV-negative patients.

Mycobacterial Diseases

The problem of coinfection with HIV and *Mycobacterium tuberculosis* is responsible for the increased incidence in tuberculosis in the United States over the past few years. This infection is discussed in detail by Reichman in another article in this journal.

Mycobacterium avium-intracellulare (MAI) is another important pathogen in patients with AIDS, often causing devastating complications in patients with advanced immunosuppression. Patients usually suffer from persistent fever, wasting, and diarrhea (39). However, MAI is rarely an important pulmonary pathogen in patients with AIDS. Its isolation in patients with overt pulmonary disease usually occurs in association with another pathogen, such as PCP.

Viral and Fungal Diseases

Fungal and viral infections of the lung may occur in individuals with HIV infection, but are less common than PCP, tuberculosis, and bacterial pneumonias. Fungal pneumonias usually occur in the setting of disseminated disease (40). Among viruses, cytomegalovirus is the most frequent isolate in specimens from the lungs of patients with HIV infection, but its role as a pathogen is uncertain. As with MAI, its presence in patients with clinically apparent pneumonitis usually occurs together with other organisms recognized to cause pulmonary disease (41, 42).

Neoplastic Diseases of the Lung

Kaposi's sarcoma (KS) is the most common malignancy in individuals with HIV infection, and the skin is the major site of involvement. However, disseminated KS may involve virtually any organ, including the lung. Patients with pulmonary KS almost always have obvious cutaneous lesions, but the lung may be the only site of disease. KS can involve the parenchyma, airways, pleura, and intrathoracic lymph nodes, leading to a diverse range of symptoms and roentgenographic findings (43, 44). Patients with parenchymal disease usually have cough and dyspnea, with diffuse or focal pulmonary infiltrates that may be nodular. Lesions in the airway can cause wheezing or hemoptysis. Pleural involvement is often associated with effusions and chest pain, and nodal disease is often apparent on chest radiographs.

The diagnosis of pulmonary Kaposi's sarcoma may be difficult to establish. The finding of typical lesions on inspection of the airways is usually regarded as diagnostic. However, the yield on bronchoscopic biopsy is low. Pleural fluid is usually exudative and sanguineous, but closed pleural biopsy is usually negative because of the focality of the lesions. Treatment regimens include chemotherapy and radiation therapy, but response rates are quite variable.

Non-Hodgkin's lymphomas also occur with increased frequency in patients with HIV infections. These tumors occasionally involve the intrathoracic lymph nodes, pulmonary parenchyma, and pleura (43). However, severe pulmonary symptoms and impairment are rare.

Noninfectious, Nonneoplastic Disorders

As the AIDS epidemic progresses, increasing numbers of patients have pulmonary disorders without an easily defined infectious or neoplastic etiology (43). These include lymphocytic interstitial pneumonitis (LIP), nonspecific interstitial pneumonitis (NIP), bronchitis obliterans organizing pneumonia (BOOP), and primary pulmonary hypertension.

LIP was described long before the first cases of AIDS, characterized by infiltration of the lung with lymphocytes and plasma cells, often in the setting of other systemic immunologic diseases. It is among the most common pulmonary complications of HIV infection in children but is relatively rare in adults (45). For unknown reasons, it is much more common in black patients, and may represent one aspect of a systematic CD-8 lymphocytic expansion. Diagnosis depends on histologic examination of lung tissue, and response to corticosteroid therapy is often dramatic.

NIP is another disorder characterized by lymphocytic infiltration of the lung, but characteristically spares the alveolar septae. This disorder has been found in individuals with and without respiratory symptoms. When symptoms are present, they are usually not severe (46). Ognibene et al. studied 24 patients with HIV with no respiratory symptoms and normal chest roentgenograms, and found that 12 of them had demonstrable NIP on transbronchial biopsy (47). In this series and others, many asymptomatic individuals with HIV infection have unexplained reductions in diffusing capacity, and NIP is a likely explanation in most.

Travis et al. recently investigated the role of HIV, Epstein-Barr virus (EBV), and cytomegalovirus (CMV) as possible etiologic agents in both LIP and NIP (48). Using in situ hybridization techniques, specimens from patients with both disorders were found to express HIV RNA, but not CMV or EBV. Using polymerase chain reaction, one of seven open lung biopsies was positive for EBV, a rate similar to results of control specimens from HIV-positive patients without lymphocytic infiltrates. These results, together with a review of histology from 46 adult HIV-infected patients with NIP and 4 with LIP, suggest that these disorders represent a spectrum of lymphoid interstitial lesions, and that HIV is the primary causative agent.

Guillon et al. found CD-8 lymphocytosis on bronchoalveolar lavage of asymptomatic HIV-infected individuals with normal chest x-rays, and more severe lymphocytosis associated with symptoms, abnormal x-rays, and abnormal pulmonary function tests (49). This "lymphocytic alveolitis" is also a manifestation of the spectrum of HIV-induced lymphoid interstitial lung disease.

A case of BOOP, without an apparent infectious or neoplastic etiology, was reported in an HIV-infected individual in 1989 (50). The diagnosis was only established with an open lung biopsy after transbronchial biopsies were inconclusive. The excellent clinical response to corticosteroids in the case report underscores the importance of establishing a specific diagnosis in patients with HIV infection and pneumonia, rather than relying on an empiric approach.

Patients who use intravenous drugs are susceptible to pulmonary hypertension because of particulate embolization granuloma formation and vasculitis. However, several HIV-positive patients are described who have pulmonary vascular lesions indistinguishable from primary pulmonary hypertension (51). The pathogenesis of this phenomenon remains unknown.

Intensive Care of Patients With AIDS

Respiratory failure is usually the immediate cause of death in patients with AIDS. An autopsy series of 216 patients with AIDS demonstrated that infection or neoplasm of the respiratory system was the immediate cause of death in 68%, followed by the central nervous system (16%), gastrointestinal tract (17%), adrenal gland (2%), kidney (2%), liver (2%), and heart (1%) (52). Consequently, respiratory failure is also the most frequent cause of admission to the intensive care unit. If the incidence of PCP declines, these causes of death may occur with different frequencies than in past years.

Treatment of respiratory failure is directed at supporting gas exchange and organ function while correcting the underlying disorder, which is usually PCP.

When treatment is postponed or ineffective, patients develop hypoxemic respiratory failure. The clinical features and chest roentgenograms in patients with severe PCP appear quite similar to the adult respiratory distress syndrome (ARDS), with hypoxemia, intrapulmonary shunting, reduced pulmonary compliance, and the x-ray appearance of diffuse alveolar infiltrates (53).

Just as severe PCP clinically resembles ARDS, the supportive treatment, including intubation, mechanical ventilation, and application of positive end-expiratory pressure, is quite similar. Continuous positive airway pressure (CPAP) delivered by face mask may also improve gas exchange without endotracheal intubation (54, 55). As the disease progresses and pulmonary compliance diminishes, pneumothorax is common. Pulmonary arterial catheterization may be employed to optimize intravascular pressures and cardiac output during positive pressure ventilation.

A smaller number of AIDS patients receive intensive care for reasons other than respiratory failure. These may include sepsis, seizures, or monitoring following invasive procedures. Diagnosis and treatment modalities are no different than for patients with other underlying illnesses. Many HIV-infected individuals are admitted to intensive care units for reasons unrelated to their infection. In most cases, their HIV serologic status is unknown at the time of admission. Therefore, ICU staff must exercise appropriate infection control precautions in caring for all patients.

Outcome. Data on outcome of intensive care of patients with AIDS have been reported since 1984. However, estimating mortality for a specific patient based on the available literature is difficult. Most published data on outcome of intensive care in patients with AIDS focuses on *Pneumocystis carinii* pneumonia (PCP) and respiratory failure. These reports indicate an improvement in survival in recent years, but the reasons for this change are not precisely known (56, 57). The use of corticosteroids as adjunctive therapy for moderate-to-severe PCP has certainly had a major impact, but other factors are important.

The criteria for selection for ICU admission may have changed. At the beginning of the AIDS epidemic, no data were available to predict outcome. Patients with respiratory failure due to PCP almost always opted for life-prolonging treatment, and physicians were likely to offer intensive care to everyone. Retrospective analyses of outcome later showed that survival rates of these patients were low, and in some centers, both physicians and patients often decided to forego this type of treatment (58). Coexisting HIV-associated conditions such as wasting, dementia, and advanced malignancy may also make ICU admission less likely now than earlier in the epidemic. Available data on outcomes do not account for these confounding factors.

There is also a bias inherent in investigators' collection of data and publications. After several initial reports of poor outcomes following mechanical ventilation for PCP, only studies demonstrating improvement in survival are likely to be carried out and published (59).

Information about outcomes of intensive care for diseases other than PCP is even more limited, because of smaller numbers of patients who were treated for a variety of conditions.

In the 1990s, the spectrum of diseases necessitating ICU management for patients with AIDS is likely to change. Antiretroviral therapies and prophylaxis for PCP will probably forestall or reduce the incidence of this infection among those who use them. Moreover, the epidemiologic shift toward patients who acquired HIV infection through injection-drug use and heterosexual contact with infected partners may lead to an increase in other life-threatening diseases, such as bacterial infections and tuberculosis. The survival rates for these populations, with a higher likelihood of diseases other than PCP, remain unknown.

References

1. Centers for Disease Control. *Pneumocystis* pneumonia–Los Angeles. MMWR 1981; 30:250–252.
2. Centers for Disease Control. HIV/AIDS surveillance report, January 1992:1–22.
3. Murray JF, Felton CP, Garay SM, et al. Pulmonary complications of the acquired immunodeficiency syndrome. Report of National Heart, Lung, and Blood Institute Workshop. N Engl J Med 1984; 310:1682–1688.
4. Golden JA, Sollitto RA. The radiology of pulmonary disease: chest roentgenography, computed tomography and gallium scanning. Clin Chest Med 1988; 9:481–495.
5. Pitchenik AE, Ganjei P, Torres A, et al. The usefulness of induced sputum in the diagnosis of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome. Am Rev Respir Dis 1987; 133:226–229.
6. Bigby TD, Margolskee D, Curtis JL, et al. The usefulness of induced sputum in the diagnosis of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome. Am Rev Respir Dis 1986; 133:515–518.
7. Kovacs JA, Ng V, Masur H, et al. Diagnosis of *Pneumocystis carinii* pneumonia: Improved detection in sputum with use of monoclonal antibodies. N Engl J Med 1988; 318:589–593.

8. Clement MJ, Luce JM, Hopewell PC. Diagnosis of pulmonary disease. Clin Chest Med 1988; 9:497–505.
9. Karpel JP, Presant D, Appel D, Bezahler G. Endotracheal lavage for the diagnosis of *Pneumocystis carinii* pneumonia in intubated patients with acquired immune deficiency syndrome. Crit Care Med 1986; 14:741.
10. Zaman MK, White DA. Serum lactate dehydrogenase levels and *Pneumocystis carinii* pneumonia. Am Rev Respir Dis 1988; 137:796–800.
11. Kagawa FT, Kirsch CM, Yenokida GG, Levine ML. Serum lactate dehydrogenase activity in patients with AIDS and *Pneumocystis carinii* pneumonia. An adjunct to diagnosis. Chest 1988; 94:1031–1033.
12. Masur H, Ognibene FP, Yarchoan R, et al. CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection. Am Intern Med 1989; 111:223–231.
13. Phair J, Munoz A, Detels R, et al. The risk of *Pneumocystis carinii* pneumonia among men infected with human immunodeficiency virus type 1. N Engl J Med 1990; 322:161–165.
14. Wharton JM, Coleman DL, Wofsy CB, et al. Trimethoprim-sulfamethoxazole or pentamidine for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome: a prospective randomized trial. Ann Intern Med 1986; 105:37–44.
15. Sattler FR, Cowan R, Nielsen DM, Ruskin J. Trimethoprim-sulfamethoxazole compared with pentamidine for treatment of *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome: a prospective, noncrossover study. Ann Intern Med 1988; 109:280–287.
16. Sattler FR, Feinberg J. New developments in the treatment of *Pneumocystis carinii* pneumonia. Chest 1992; 101:451–457.
17. Medina I, Mills J, Leoung G, et al. Oral therapy in the acquired immunodeficiency syndrome—a controlled trial of trimethoprim-sulfamethoxazole versus trimethoprim-dapsone. N Engl J Med 1990; 323:776–782.
18. Black JR, Feinberg J, Murphy R, Fass RJ, Carey J, Sattler FR. Clindamycin and primaquine as primary treatment for mild and moderately severe *Pneumocystis carinii* pneumonia in patients with AIDS. Eur J Clin Microbiol 1991; 10:204–207.
19. Montgomery AB, Debs RJ, Luce JM, et al. Aerosolized pentamidine as sole therapy for *Pneumocystis carinii* pneumonia in patients with acquired immunodeficiency syndrome. Lancet 1987; 2:480–483.
20. Conte JE, Hollander H, Golden JA. Inhaled or reduced-dose, intravenous pentamidine for *Pneumocystis carinii* pneumonia. Ann Intern Med 1987; 107:495–498.
21. Miller RF, Godfrey-Faussett P, Semple SJG. Nebulized pentamidine as treatment for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. Thorax 1989; 44:565–569.
22. The National Institutes of Health–University of California Expert Panel for Corticosteroids as Adjunctive Therapy for *Pneumocystis* Pneumonia. Consensus statement on the use of corticosteroids as adjunctive therapy for *Pneumocystis* pneumonia in the acquired immunodeficiency syndrome. N Engl J Med 1990; 323:1500–1504.
23. Bozzette SA, Sattler FR, Chin J, et al. Corticosteroids as adjunctive therapy in severe *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. N Engl J Med 1990; 323:1451–1456.
24. Clement M, Edison R, Turner J, et al. Corticosteroids as adjunctive therapy in severe *Pneumocystis carinii* pneumonia. A prospective placebo-controlled trial [Abstract]. Am Rev Respir Dis 1989; 139(suppl):A250.
25. Montaner JSG, Lawson LM, Levitt N, et al. Corticosteroids prevent early deterioration in patients with moderately severe *Pneumocystis carinii* pneumonia and the acquired immunodeficiency syndrome (AIDS). Ann Intern Med 1990; 133:14–20.
26. Centers for Disease Control. Guidelines for prophylaxis against *Pneumocystis carinii* pneumonia for persons infected with human immunodeficiency virus. MMWR 1989; 38(S-5):1–9.
27. Fischl MA, Dickinson GM, La Voie L. Safety and efficacy of sulfamethoxazole and trimethprim chemoprophylaxis for *Pneumocystis carinii* pneumonia in AIDS. JAMA 1988; 259:1185–1189.
28. Wormser GP, Horowitz HW, Duncanson FP, et al. Low dose intermittent trimethoprim-sulfamethoxazole for prevention of *Pneumocystis carinii* pneumonia in patients with human immunodeficiency virus infection. Arch Intern Med 1991; 151:688–692.
29. Montgomery AB, Debs RJ, Luce JM, et al. Selective delivery of pentamidine to the lung by aerosol. Am Rev Respir Dis 1988; 137:477–478.
30. Leoung GS, Feigal DW, Montgomery AB, et al. Aerosolized pentamidine for prophylaxis against *Pneumocystis carinii* pneumonia. The San Francisco Community prophylaxis trial. N Engl J Med 1990; 323:769–775.
31. Abd AG, Nierman DM, Ilowite JS, Pierson RN, Jr., Bell AL, Jr. Bilateral upper lobe *Pneumocystis carinii* pneumonia in a patient receiving pentamidine prophylaxis. Chest 1988; 94:329–331.
32. Jules-Elysee KM, Stover DE, Zaman MB, Bernard EM, White DA. Aerosolized pentamidine: effect on the diagnosis and presentation of *Pneumocystis carinii* pneumonia. Ann Intern Med 1990; 112:750–757.
33. Murray JF, Mills J. Pulmonary infectious complications of human immunodeficiency virus infection. Am Rev Respir Dis 1990: 1356–1372, 1582–1599.
34. Chaisson RE. Bacterial pneumonia in patients with human immunodeficiency virus infection. Semin Resp Inf 1989; 4:133–138.
35. Polsky B, Gold JWM, Whimby E, et al. Bacterial pneumonia in patients with the acquired immunodeficiency syndrome. Ann Intern Med 1986; 104:38–41.
36. Lane HC, Masur H, Edgar LC, Whalen G, Rook AH, Fauci AS. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N Engl J Med 1983; 109:453–458.
37. Magnenat J-L, Nicod L, Aukenthaler R, Junod A. Mode of presentation and diagnosis of bacterial pneumonia in human immunodeficiency virus-infected patients. Am Rev Respir Dis 1991; 144:917–922.
38. Pesola GR, Charles A. Pneumococcal bacteremia with pneumonia. Mortality in acquired immunodeficiency syndrome. Chest 1992; 101:150–155.
39. Hawkins CC, Gold JW, Whimby E, et al. *Mycobacterium avium* complex infections in patients with the acquired immunodeficiency syndrome. Ann Intern Med 1986; 105:184–188.
40. Fels AOS. Bacterial and fungal pneumonias. Clin Chest Med 1988; 9:449–457.
41. Wallace JM. Pulmonary infection in human immunodeficiency disease: viral pulmonary infections. Semin Resp Inf 1989; 4:147–154.
42. Jacobson MA, Mills J, Rush J, et al. Morbidity and mor-

tality of patients with AIDS and first-episode *Pneumocystis carinii* pneumonia unaffected by concomitant pulmonary cytomegalovirus infection. Am Rev Respir Dis 1991; 144:6–9.

43. White DA, Matthay RA. Noninfectious pulmonary complications of infections with the human immunodeficiency virus. Am Rev Respir Dis 1989; 140:1763–1787.
44. Ognibene FP, Shelhamer JH. Kaposi's sarcoma. Clin Chest Med 1988; 9:459–466.
45. Teirstein AS, Rosen ML. Lymphocytic interstitial pneumonia. Clin Chest Med 1988; 9:467–471.
46. Suffredini AF, Ognibene FP, Lack EE. Non specific interstitial pneumonitis; a common cause of pulmonary disease in the acquired immunodeficiency syndrome. Ann Intern Med 1987; 107:7–13.
47. Ognibene FP, Masur H, Rogers P, et al. Nonspecific interstitial pneumonitis without evidence of *Pneumocystis carinii* in asymptomatic patients infected with human immunodeficiency virus (HIV). Ann Intern Med 1988; 109:874–879.
48. Travis WD, Fox CH, Devaney KO. Lymphoid pneumonitis in 50 adult HIV-infected patients: lymphocytic interstitial pneumonitis versus nonspecific interstitial pneumonitis. Hum Pathol (in press).
49. Guillon JM, Antran B, Denis M, et al. Human immunodeficiency virus-related lymphocytic alveolitis. 1988; 94:1264–1270.
50. Allen JN, Wewers MD. HIV-associated bronchiolitis obliterans organizing pneumonia. Chest 1989; 96:197–198.
51. Coplan NL, Shimony RY, Ioachim HL, et al. Primary pulmonary hypertension associated with human immunodeficiency viral infection. Am J Med 1990; 89:96–99.
52. Klatt EC. Diagnostic findings in patients with acquired immune deficiency syndrome (AIDS). J AIDS 1988; 1:459–465.
53. Maxfield RA, Sorkin B, Fazzini EP, Rapoport DM, Stenson WM, Goldring RM. Respiratory failure in patients with acquired immunodeficiency syndrome and *Pneumocystis carinii* pneumonia. Crit Care Med 1986; 14:443–449.
54. DeVita MA, Greenbaum DM. The critically ill patient with the acquired immunodeficiency syndrome. In: Shoemaker WC, Ayres S, Grevik A, Holbrook PR, Thompson WL, eds. Textbook of critical care medicine. Philadelphia: W. B. Saunders Company, 1989;862–869.
55. Gregg RW, Friedman BC, Williams JF, et al. Continuous positive pressure by face mask in *Pneumocystis carinii* pneumonia. Crit Care Med 1990; 18:21–24.
56. Wachter RM, Russi MB, Bloch DA, Hopewell PC, Luce JM. *Pneumocystis carinii* pneumonia and respiratory failure in AIDS: improved outcomes and increased use of intensive care units. Am Rev Respir Dis 1991; 143:251–256.
57. Friedman Y, Franklin C, Freels S, Weil MH. Long term survival of patients with AIDS, *Pneumocystis carinii* pneumonia, and respiratory failure. JAMA 1991; 266:89–92.
58. Wachter RM, Luce JM, Turner J, Volberding P, Hopewell PC. Intensive care of patients with the acquired immunodeficiency syndrome: outcome and changing patterns of utilization. Am Rev Respir Dis 1986; 134:891–896.
59. Wachter RM, Luce JM. Intensive care for patients with *Pneumocystis carinii* pneumonia and respiratory failure: are we prepared for our new success? Chest 1989; 96:714–715.

Grand Rounds

HIV Infection:

The Dominant New Face of Tuberculosis

Lee B. Reichman, M.D., M.P.H.

In New York City in 1904, the five-year survival rate for tuberculosis was 50%. People with tuberculosis typically took the fresh-air cure; they lay on the roof in the open air. So some people with tuberculosis do get better without treatment.

Today, however, tuberculosis is burgeoning into a huge worldwide problem. A third of the world's population is infected with the tubercle bacillus. Up to 10 million new active cases of tuberculosis and 2⅓ million deaths due to tuberculosis occur every year, constituting 7% of all deaths from any cause worldwide—and 26% of all potentially preventable deaths.

In Africa, 5 million people have been HIV infected. Many of them live where tuberculosis is prevalent, and, reflecting that, in the late 1980s African countries experienced an increase in tuberculosis ranging from 3% to 31%. This is embarrassing to medicine, because we know most of the things we need to know about tuberculosis: transmission, pathogenesis, case finding, prevention, and specific therapy.

In the United States, since the collection of cases of tuberculosis started about 1955, the number of cases dropped each year about 5% until 1984, when it leveled off and began to rise. From 1988 to 1989 it rose 5%; from 1989 to 1990, it rose 9.4%. In specific areas such as New York City it doesn't fall, but continues to rise; in 1990 it was 38%. In Newark, from 1988 to 1989, it rose 35%; as it did in Watts in Los Angeles. In the state of New Jersey it rose 20% last year. These figures add up to a gigantic epidemic. Data from the Centers for Disease Control show the cases to be rising among blacks and Hispanics aged 20 through 40 year by year; in non-Hispanic whites it is not rising, and most victims are in elderly.

This epidemic rise is a reflection of the AIDS epidemic. Even in AIDS history, which only goes back about 10 or 11 years, tuberculosis with AIDS is relatively recent. The relationship was first described in Haitians by the Florida group in 1984 (1). Our group described it in intravenous drug abusers in 1986 (2). Tuberculosis is being increasingly recognized in those groups who have a high incidence of HIV infection. The risk of tuberculosis in people who have AIDS is higher than any previously described risk factor that predisposes to tuberculosis. This is important to recognize because tuberculosis is one of the few contagious respiratory diseases that occurs in patients with HIV infection and AIDS, and is both treatable and potentially preventable.

The distinction between HIV infection and ARC and AIDS is largely one of definition, as published in Morbidity and Mortality Report of the CDC.

Clinical Manifestations

The clinical picture of tuberculosis in HIV-infected people is not the classic picture; it is "weird"; it is out of the ordinary.

In 1980 the incidence of extrapulmonary tuberculosis in the United States was 14%; in 1984 it was 16%. In the city of Newark it was 17% in 1980, 27% in 1984. In our first 29 cases of tuberculosis and HIV infection in 1985–1986 the incidence of extrapulmonary tuberculosis in cases defined as AIDS by CDC criteria was 73%.

The syndrome may include acute pericarditis with tamponade leading to a chronic pericardiocutaneous sinus; abscess of the chest wall; osteo-

---

Adapted from the author's Grand Rounds in Medicine presentation at Mount Sinai Medical Center on February 21, 1991. From the Pulmonary Division, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, University Hospital. Address reprint requests to the author, Director of the Pulmonary Division at the University of Medicine and Dentistry of New Jersey, New Jersey Medical School, University Hospital, 150 Bergen Street, Newark, NJ 07103-2425.

myelitis of the rib; recurrent rectal abscess; multiple brain abscess; chronic tuberculomas simulating brain tumors; tuberculosis of the wrist, ankle, testes, or large intestine with perforation initially appearing as an acute abdomen. Any one, two, or three such patients in a chest clinic that sees a lot of tuberculosis would not be exciting, but you see case after case in intravenous drug users who are ultimately defined to be HIV-infected or to have AIDS. Extrapulmonary tuberculosis in an AIDS-infected person became an AIDS-defining diagnosis in CDC criteria in 1988. This is indeed "weird" tuberculosis, amounting to a new syndrome, because it was not seen as extensively prior to the AIDS epidemic.

The chest x-rays are also strange. Study of our first 29 cases (2) revealed classical upper lobe infiltrates such as one sees in tuberculosis in six, miliary in four, nodular in three, interstitial infiltrates in three, hilar adenopathy in six, mediastinal adenopathy in four, peritracheal adenopathy in one, pleural effusions in five, and cavitary lesions in five. Two of the cavities were air fluid levels, uncommon in tuberculosis.

Some examples, all intravenous drug users who ultimately had AIDS or were HIV infected if that occurred after the HIV test became available:

Case 1. A man with pericardial effusion who had a pericardial window which cultured out *Mycobacterium tuberculosis* was treated but drained through the chest wall on treatment.

Case 2. This patient had right upper lobe, more classical-looking cavitary tuberculosis and was given antituberculous medication. A few months later, he had acute abdomen; operation showed a fistula from the gut, and the fistula itself grew out *M. tuberculosis.*

Case 3. A patient with back pain whose scout film had a soft tissue shadow; a bone scan which lit up; a myelogram which showed a blockage; CT of the vertebra which showed an abscess, which on needle biopsy grew out *M. tuberculosis.*

Case 4. A patient with pain and swelling of the wrist in 1984; synovial biopsy showed acid-fast bacilli that grew out *M. tuberculosis;* his compliance with a regimen of antituberculous medication was not good. A year later, he came in with upper lobe cavitary tuberculosis. So it goes from extrapulmonary to pulmonary sometimes.

Case 5. A patient reported on separately (3) had two cavities in the left upper lobe in 1985. He had positive sputum smears and cultures. He was placed on a Centers for Disease Control interinstitutional cooperative trial of isoniazid-rifampin-pyrazinamide for six months, versus isoniazid-rifampin for nine months; he was on the study regimen. He took his medication without problems, except for a period of four weeks during a trip in the middle of the regimen. He apologized to us and said he would be good and take his medicine, so we extended the regimen to seven months, which he took. In his x-ray at the end of the seven months, in December 1985, the cavities were closed, his sputum was negative, he had gained weight, he had no more sputum, and he felt fine. We counted him as a cure.

In April 1986 on a routine follow-up visit for the controlled clinical trial, he complained of pain and swelling of the testicle. On testicular ultrasound he had an echo-free space; his testicular scan lit up; x-ray at that time was unchanged from the prior x-ray, but an orchiectomy revealed *M. tuberculosis* of the testicle, sensitive to the same antibiotics that he was on previously which cured his lung lesion.

That same month we were preparing page proofs for our article (2) saying that weird extrapulmonary tuberculosis is a harbinger of HIV, and we decided to ask if he had any risk factors. He had used drugs 20 years previously, but denied any other behavior accountable as a risk. We did test for HIV; it was positive, his T cell count was about 50. We reinstituted him on antituberculous medication, the same medication he was on; he did very well, kept his appointments until January 1987, when he developed *Pneumocystis carinii* pneumonia. He died; autopsy showed *Pneumocystis,* no evidence of tuberculosis.

On the basis of that experience we posed the question (3) whether people whose immune system isn't helping them need medication for longer periods than the average six months for tuberculosis. Michael Iseman, in an accompanying editorial (4), noted that although this patient missed three weeks of medication, he got treated as well as most people get treated. So the question remained open.

Case 6. A patient who consulted us for a headache had about four or five different abscesses in the brain; needle biopsy showed *M. tuberculosis.* We put him on medication. He survived a few years, till he died of *Pneumocystis.*

Case 7. A woman seen in 1983 in another hospital was told she had an incurable brain tumor. She came to our hospital; we did a needle biopsy, grew out *M. tuberculosis,* placed her on antituberculous medication. She took her medicine fairly well—she was not a perfect complier, because she was alcoholic—and she's never been on AZT. She did very well. Her case was included in a series on central nervous system tuberculosis published in 1986 (5). In January 1991 her T cells numbered 500.

To summarize the clinical manifestations of tuberculosis and HIV infection: early in HIV infection, the skin test is positive, adenopathy is unusual, pulmonary distribution is upper lobe, cavitation is present and extrapulmonary distribution is about 15%. Late in HIV infection, the skin test turns negative, adenopathy is common, pulmonary distribution becomes more middle lobe and lower lobe, the cavitation is absent, and the extrapulmonary distribution becomes more than 50%.

When these unusual manifestations occur, the diagnosis is more difficult to make; as a result one has to have a high index of suspicion. In other words, the usual methods used to make the diagnosis are often unproductive. For instance, tuberculin skin tests were negative in 27 of our first 29 patients in Newark, and in 14 of 23 patients described from San Francisco who had tuberculosis. Sputum was positive in only 7 of our first 29 cases in Newark, and in 26 of the first 35 cases described from San Francisco. Treatment has to be aggressive and invasive, and that means culturing everything for tuberculosis: sputum, bronchial washings, transbronchial and open lung biopsies, lymph nodes, liver, synovium, bone marrow biopsies, urine, blood, cerebrospinal fluid, needle aspirations and excisional biopsies of brain mass lesions, wound discharges, colonoscopic and laparoscopic biopsies. The rule is that in any biopsy, make sure some of the material goes into transport medium or saline so it can go to the bacteriology lab. A sample placed in formaldehyde is rendered useless for culture.

So the unusual clinical features emphasize the importance of maintaining a high index of suspicion for tuberculosis; those who care for HIV-positive patients must be informed and reminded of the frequent uncharacteristic presentation of tuberculosis, because the penalty for failing to make the diagnosis of tuberculosis and to institute appropriate management can result in the death of the patient. More important, perhaps, is tuberculosis infection of the contacts, including the caregivers.

Remember that tuberculosis is transmitted as a droplet aerosol infection and it may be transmitted any time you help the patient to cough. Of course, it takes a long time to spread tuberculosis; Riley estimated that six months of eight-hour-a day contact is needed to produce a 50% chance of catching it, but anyone in close proximity to someone with undiagnosed tuberculosis who is aerosolizing a lot is at risk.

Bronchoscopies are extensively used to diagnose infections in HIV-infected people. Many people requiring intubation have HIV-related tuberculosis infections which may or may not be diagnosed. Bronchoscopy almost always induces coughing, artificial airways are always suctioned, and the highest rate of tuberculosis infectivity yet recorded occurred during bronchoscopy in endotracheal intubation in an intensive care unit.

Aerosolized pentamidine always induces coughing and can thereby facilitate the transmission of any infection that the person who's getting the aerosolized pentamidine has. Anybody who is sharing the same breathing space is at risk of getting infected by whatever the person coughing has. That means other HIV-infected contacts but also healthy staff and treatment center and household contacts if the treatment are given at home. It's common sense that the potential transmission of tuberculosis depends on the prevalence of the tuberculosis infection in the HIV-infected population that's being served but also the fresh-air room ventilation, the number of droplet nuclei that are generated by the patient, and the duration of the exposure.

In a recently published study by Charlotte Malasky et al. (6) we looked at the risk of tuberculosis transmission to staff, including pulmonary fellows and infectious disease fellows in the top 25 cities in the United States. We assumed the demographics would be the same, but pulmonary fellows do bronchoscopies and intubations, whereas infectious disease fellows do not. Among pulmonary fellows, 7 of 62 converted their tuberculin test; among infectious disease fellows, the rate was 1 in 42, statistically a significant difference. We assumed the reason for the difference was that pulmonary fellows do invasive procedures that generate aerosols, and they may or may not have used masks. They are at risk. But more important for public health are recent reports of transmission of multidrug-resistant tuberculosis—47 cases of people infected with resistant tuberculosis in Jackson Memorial Hospital. The key question arises: What prophylaxis can we give somebody infected with a tubercle bacillus resistant to many drugs? As yet we have no clear answer.

Treatment of Tuberculosis

Another *Mycobacterium, M. avium,* often infects HIV-infected people, but since no clinical or laboratory criteria other than culture differentiates between *M. tuberculosis* and *M. avium*, intracellular chemotherapy with three antituberculosis drugs should be initiated when acid-fast bacilli are seen. [New York City Department of Health is now recommending four drugs.] Tuberculosis is transmissible but curable even in AIDS patients.

The recommended regimen is isoniazid at 300 mg/day and rifampin 600 mg/day for six months, and pyrazinamide 20–30 mg/kg—1½–2 g/day for the initial two months. Treatment should continue for a minimum of nine months. In the absence of HIV infection, six months' treatment with this regimen—pyrazinamide for the first two months, isoniazid and rifampin for six months—is the standard regimen preferred by the U.S. Public Health Service. In the presence of HIV infection, they suggest treating for six months after culture conversion, generally nine months. We expect that drug susceptibility is not affected by HIV infection, and most patients, even those who have severe immunosuppression or AIDS with opportunistic infection, seem to respond to antituberculosis treatment.

There are four populations of tubercle bacilli that differ in rate of bacterial growth. Population A are continuously growing; isoniazid is the best treatment. Population B are intracellular organisms in the macrophage, in an acidic medium, whose growth is retarded; pyrazinamide enters the cells and is thus the best treatment. Population C are slow-growing organisms that have spurts of metabolism; during the growth spurt, rapid-acting rifampin is best. Population D are dormant bacilli that do not grow unless they have a rapid growth spurt.

Choosing drugs that have such specific actions leads to two phases of the treatment of tuberculosis: bactericidal, the initial intensive chemotherapy directed toward rapid destruction of large multiplying populations of tubercle bacilli (standard therapy since the 1950s: isoniazid); and sterilizing or maintenance chemotherapy, directed at dormant bacilli during intermittent spurts of metabolic activity and at intracellular bacilli. Successful chemotherapy for tuberculosis consists of putting these two phases of treatment together. For bactericidal effect, isoniazid-rifampin are best; for sterilizing effect, rifampin-pyrazinamide are best.

Ethambutol is not mentioned, because it is a bacteriostatic effective in guarding against resistant organisms but not in initial treatment for tuberculosis, unless one suspected resistant disease. If I had tuberculosis, I would want to be treated with isoniazid-rifampin-pyrazinamide for two months and isoniazid-rifampin for an additional four months. If I were HIV-infected, I'd extend the isoniazid-rifampin to a total of nine months. That would cure over 99% of tuberculosis cases.

HIV Infection and Tuberculosis

In the United States about 10 million people are infected with the tubercle bacillus. About 1½ million people are infected with HIV. The overlap between these groups are the people whose immune system is not protecting them from breakdown of their tuberculosis; they are at strong risk of getting tuberculosis. The degree of overlap differs geographically; in a rural Western area in the United States, the tuberculosis-infected group and the HIV-infected group do not overlap much, so tuberculosis is not common. But in an inner city in the United States—Harlem in New York City, or Watts in Los Angeles, or Newark—the overlap is much greater. In Africa or Haiti, the overlap is huge.

So diagnosing tuberculous infection in people who are HIV-infected is imperative because we can treat them. A Mantoux skin test with five units of PPD should be administered, but the big problem with tuberculin testing of patients with HIV infection is that some have false negative reactions because of their immunosuppression. The proportion of HIV-infected people with tuberculosis who have negative tuberculin skin tests can be determined by the length of the interval between the diagnosis of tuberculosis and the diagnosis of the AIDS. As the number of T cells goes down, the likelihood of immunosuppression, and of a negative skin test, rises. Because the risk of tuberculosis is large in people with HIV infection, the sensitivity of the test is much more important than the specificity, and the Centers for Disease Control criterion is that a tuberculin reaction of 5 mm induration in an HIV-infected individual is indicative of tuberculous infection.

But the problem is that physicians don't like to give preventive therapy. CDC program management data show that isoniazid preventive therapy was being prescribed up to 1981, and then stopped. In 1981 a decision analysis study concluded that the risk of giving isoniazid to asymptomatic young adult males was greater than the risk of their getting tuberculosis, so physicians should not give isoniazid. Although George Comstock in an editorial concluded that the decision analysis data were wrong, everybody read the decision analysis, no one read the editorial, and everyone stopped using preventive therapy. In 1986, Comstock in another editorial lamented the lack of preventive therapy in general. He pointed out an inherent and unfortunate tendency for prevention to be discouraged because preventive effects are rarely visible and dramatic, because something prevented is a nonevent, identified only statistically, whereas bad effects of preventive measures are readily recognizable, because they tend to occur close to the time of the preventive procedure even if they're only coincidental. He said that the risk of tuberculosis in an infected person is not great and occurs over the total remaining lifespan, while the risk of any preventive treatment occurs during a relatively short period when the medication is taken, so it's easy for the physician to avoid the immediate risk of treatment rather than the distant threat of disease, and physicians tend to accept the improbable risk of being sued for tuberculosis that developed because preventive therapy is not recommended rather than face the immediate risk of a lawsuit deriving from the side effects of treatment.

Risk Factors for Tuberculosis. Tuberculosis in an infected person becomes active in people with traditional risk factors. For infection less than a year, absolute risk is 10.4 per thousand per year; for infection more than seven years, it is 0.7; for someone who has silicosis, it is 30. But for someone who has HIV infection, the absolute risk is 149 per thousand, and for someone who has AIDS, the relative risk is 170—huge, mind-boggling risks, compared to the group of asymptomatic young adult males, the population discussed in the 1981 article on decision analysis that so discouraged preventive isoniazid therapy.

A 1989 prospective study by Selwyn et al. (7) of 513 intravenous drug users found 8 who were HIV positive out of 215 who got active tuberculosis, and none who were HIV-negative. They found 14%—7 of 49—had prior positive tuberculin tests, and none had prior known negative tuberculin tests. The single subject who had a negative tuberculin test had anergy and oral candidiasis nine months prior to his tuberculosis, settling the diagnosis. Of the patients who received isoniazid preventive therapy, none (of 13) got tuberculosis; of patients who did not receive isoniazid, 19% got tuberculosis. These results are not statistically significant, but they are suggestive.

We physicians should affirm that we should give isoniazid preventive therapy, we want to give isoniazid preventive therapy, and this is the critical group to receive isoniazid preventive therapy. The problem is many in this critical group have negative tuberculin tests, and how can we give them isoniazid preventive therapy if their tuberculin tests are negative?

Following the isoniazid decision-analysis controversy that began in 1981, many models have been used to conclude variously that giving isoniazid or not giving isoniazid adds five days of life or subtracts five days of life. Using decision analysis, my group (8) asked, Should isoniazid be prescribed for HIV-positive patients in whom tuberculin testing itself is a poor marker? We studied 35-year-old intravenous drug users in high-risk areas with probable anergy, and we used assumptions and parameter estimates that were highly conservative anti-isoniazid.

We found the number of days of life gained or lost as a result of prescribing isoniazid as preventive therapy were, with a positive tuberculin test, 200 to 300 days of life added; without a tuberculin test, 60 or 80 days of life added; with a negative tuberculin test (except for black women, who don't handle isoniazid well), and using the most anti-isoniazid data we could find in the literature, 10 days of life added.

Thus the risk of tuberculosis is so strong that physicians probably should give isoniazid to 35-year-old intravenous drug abusers. Intravenous drug abuse is priority number one; it is the most common AIDS risk factor among the tuberculosis–AIDS patients nationwide. It is the most likely reason for the explosive increase in tuberculosis in New York City, where 50%–60% of intravenous drug users are HIV seropositive and 20%–30% of the minority intravenous drug users have tuberculous infection. The potential for rapid, explosive tuberculosis epidemics exists in many other cities with a huge number of intravenous drug users promoting rapid HIV transmission. Methadone clinics are supposed to screen for tuberculosis, but do not always do so. In New York City, HIV co-infection is doubling the number of tuberculosis cases.

Nationwide the risk of tuberculosis for HIV-positive persons is about 7% to 10% per year. For HIV-negative persons, it is 5% or 10% lifetime. There are 1.3 million intravenous drug users in the U.S., raising the possibility of 43,000 new tuberculosis cases—currently there are about 25,000 tuberculosis cases in the United States.

Prisons pose an additional problem. There the HIV infected and the tuberculosis infected live in close contact, and the number of cases of tuberculosis has risen hugely.

HIV infection spreads with difficulty in risk groups, and from risk groups to others. Tuberculosis can spread to people who share the same air for a variable length of time. With an increase in HIV cases documented and an increase in AIDS assured, an increase in tuberculosis transmissible from risk groups to groups not at risk must occur and has already been noted. Tuberculosis is back, perhaps with a vengeance. But it is still curable and still controllable, given dedication, care, and resources.

Questions and Answers

Question: Do you give isoniazid to the dually infected person, granted their life expectancy may not be that long? But how do you expect the isoniazid to last after you stop using the isoniazid?

Reichman: A good question. Nobody knows. Presumably, what the isoniazid does is reduce but not eliminate the organisms that lead to the infection. Infection without disease is a few organisms. That's why one drug is effective alone. It reduces, it doesn't eliminate. The problem is, no one has studied the question you're asking. Lifelong isoniazid has not been shown to be beneficial, and the Centers for Disease Control recommends 12 months isoniazid. The recommended preventive therapy for isoniazid is six months in everybody, 12 months in HIV-infected persons. Isoniazid for 12 months should eliminate most of the organisms, which we hope will do something. But we don't know for sure.

How do we get intravenous drug users who are HIV-infected to take their isoniazid for 12 months? Everyone says, give all HIV-infected people with tuberculous infection INH. This is similar to use of zidovudine in individuals with T-cell counts below 500; AZT for life. Let them take five pills a day for life; HHS says that, the secretary says that, everybody says that. But no one says how we are going to get people to take these medications. Some patients are dedicated, they have beepers which go off, and they take their medication. But most people are not that dedicated; they don't take their hypertension medication, they don't take their insulin, they don't take their asthma medicine, they don't take their tuberculosis medicine, they don't take their AZT. This is the kind of problem that should be looked into because it's economically beneficial to prevent tuberculosis—we prevent hospitalizing, we won't need to use expensive drugs. Preventing AIDS would have tremendous economic effects. Unfortunately people are not interested in looking at these problems.

Question: If you have a population that's not compliant, do you run the risk of developing a resistant population by giving INH?

Reichman: That doesn't generally happen if you have few organisms. Remember, we're using preventive therapy, we're treating for few organisms. The way you get resistant organisms is, the organisms mutate in the presence of the antibiotic. If you have a large population and you give the antibiotic, the organisms mutate and then the antibiotic selects out the resistant mutants. Presumably, with isoniazid, the mutation rate is something like, approaches the figure, one out of 100,000 organisms per cell division, and someone with tuberculosis infection without disease has less than 100,000 organisms—a focus in the lung, say, not visible on x-ray, or extrapulmonary tuberculosis without a big abscess and thus with few organisms. So the problem is not gigantic.

Resistance also develops by taking one drug without another in a large population, so if the physician is in doubt about what the population is, he or she can give two or more drugs.

Question: Previously you indicated that you would treat patients under 35 years with INH for a year; but patients older than age 35 you wouldn't. Does this still hold?

Reichman: No. The Centers for Disease Control guidelines changed in May 1990 (9). The easiest way to remember is, above age 35, treat only those with a risk factor, and HIV infection is the strongest but not the only one; below age 35, treat everybody with no risk factor and a large reaction.

The CDC skin test size guide has also changed: preventive therapy is most important for 5 mm of induration; intermediate for 10 mm of induration. For people at low risk—say, a healthy resident who converted his skin test at age 30, who may get tuberculosis but has a strong immune system and may not have a problem—15 mm of induration is the signal for preventive therapy.

Question: Is there any experience with BCG immunized populations who have BCG and then imposition of HIV?

Reichman: That's something I'm concerned about. I was so concerned I wrote a letter published in *JAMA* a couple of years ago (10) and there were no comments. The letter said something like—there was a disease once called *M. avian intracellulare,* and it was a curiosity, and every year a hospital like this would see one or two cases, and a hospital somewhere else would see one or two cases, and it was difficult to treat. Then the AIDS epidemic came along, and MAI became a huge disease in an AIDS-defining diagnosis, because people were infected with it, didn't have problems, their immune system went out, and all of a sudden they got *M. avium intracellulare,* it was difficult to treat.

The situation is similar, I would think, in places like Africa, where many people are given BCG in infancy, and HIV infection is rife. Since the AIDS epidemic began, huge epidemics of disseminated BCG have not occurred even though the World Health Organization, as part of its expanded program in immunization, gives BCG and five or six other vaccines to all the infants in certain areas. Why? About five or six cases have been reported, usually in older people—BCG has been used for bladder tumors, for example. I think one of three reasons is obtaining. Reason one: the diagnosis isn't being made. In Africa, the diagnosis is made by bacilloscopy: they look at the smear, they don't culture the organisms, and they say, Gee, this kid's got tuberculosis, we'll treat him for tuberculosis, but they don't know that it's *M. bovis*. Reason two: it hasn't happened yet, but it's going to happen. Reason three: maybe there are no organisms in the BCG. These vaccines are used all over the world—they're supposed to be attenuated, maybe they're so attenuated they don't even grow in people.

I don't know what the answer is, but it seems to me that if the World Health Organization is continuing to give BCG and there's a huge AIDS epidemic, sooner or later there may be an epidemic.

Question: It seems to me that a non-skin-test method of diagnosing tuberculosis would be helpful. I remember Rosalyn Yalow's radioimmunoassay. Is that method being looked at?

Reichman: People are looking at all sorts of things for that because the epidemic has made tuberculosis sexy again. So there's money in tuberculosis. NIAID is putting money in tuberculosis, and the Division of Lung Disease is even interested in putting money in tuberculosis research, and it's become commercially viable—but not in the United States. In this country there are only 25,000 cases, and companies won't do something if they're only going to treat or diagnose 25,000. But as the number of infected people rises and more are at risk, perhaps interest will strengthen. Nothing I know of is on the horizon. Remember, the place where this is going to become important is Africa, and radioimmunoassay would be difficult there; in the developing world it's a far way off.

References

1. Pitchenik AE, Burr J, Suarez M, et al. Tuberculosis, atypical mycobacteriosis and the acquired immunodeficiency syndrome among Haitian and non-Haitian patients in South Florida. Ann Intern Med 1984; 101:641–645.
2. Sunderam G, McDonald RJ, Maniatis T, Oleske J, Kapila R, Reichman LB. Tuberculosis as a manifestation of the acquired immunodeficiency syndrome. JAMA 1986; 256:362–366.
3. Sunderam G, Mangura BT, Lombardo J, Reichman LB. Failure of "optimal" four-drug short course tuberculosis chemotherapy in a compliant human immunodeficiency virus patient. Am Rev Respir Dis 1987; 136:1475–1478.
4. Iseman MD. Is standard chemotherapy adequate in tuberculosis patients infected with HIV? (editorial). Am Rev Respir Dis 1987; 136:1326–1327.
5. Bishburg E, Sunderam G, Reichman LB, Kapila R. Central nervous system tuberculosis with the acquired immunodeficiency syndrome and its related complex. Ann Intern Med 1986; 105:210–213.
6. Malasky C, Jordan T, Potulski F, Reichman LB. Occupational tuberculous infections among pulmonary physicians in training. Am Rev Respir Dis 1990; 142:505–507.
7. Selwin PA, Hartel D, Lewis VA, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. N Engl J Med 1989; 320:456–450.
8. Jordan TJ, Lewit EM, Montgomery RL, Reichman LB. Isoniazid as preventive therapy in HIV infected intravenous drug abusers. A decision analysis. JAMA 1991; 265:2987–2991.
9. Centers for Disease Control. Screening for tuberculosis and tuberculous infection in high risk populations and the use of preventive therapy for tuberculous infection in the United States. MMWR May 18, 1991; 39:1–12 #RR8.
10. Reichman LB. Why hasn't BCG proved dangerous in HIV-infected patients? JAMA 1989; 261:3246.

When you don't know all
the medicines your patients are taking,
protecting their health
is a crap shoot.

Counseling patients about all their medicines improves their odds of getting well and staying well.

EVERYONE WINS WHEN YOU TALK

Send me a *free* Medicine Counseling Kit.

Name

Organization

Street

City State Zip

Mail to:

National Council on Patient Information
and Education
666 Eleventh Street, NW, Suite 810
Washington, DC 20001
To fax your request -- (202) 638-0773

this publication is available in microform

Please send me additional information.

University Microfilms International

300 North Zeeb Road
Dept. P.R.
Ann Arbor, MI 48106
U.S.A.

18 Bedford Row
Dept. P.R.
London, WC1R 4EJ
England

Name

Institution

Street

City

State Zip

Instructions for Authors

THE MOUNT SINAI JOURNAL OF MEDICINE, established in 1934 as *The Journal of the Mount Sinai Hospital,* is a peer-reviewed general medical journal. It is indexed or abstracted in *Index Medicus, Current Contents, Science Citation Index, Hospital Literature Index, International Nursing Index, Excerpta Medica, Chemical Abstracts, Biological Abstracts,* and major databases. Theoretical and basic science articles for the clinician, reviews, reports of clinical or research experience, articles on any subject affecting the practice of medicine, short notes in any specialty, book reviews, letters, and articles by medical students are welcome. The *Journal* is a participant in the Agreement on Uniform Requirements for Manuscripts Submitted to Biomedical Journals. The *Journal* aims at responding to authors of manuscripts within 8 weeks of date of submission.

Communications. Address correspondence on manuscripts review to: Managing Editor, *The Mount Sinai Journal of Medicine,* Box 1094, 19 E. 98th Street, 4D, New York, NY 10029-6574. Phone: (212) 241-6108; FAX: (212) 722-6386.

Copyright. The publisher reserves copyright and renewal rights on all published material, and such material may not be published elsewhere without written permission of the publisher. When submitting a manuscript, include the following assignment of copyright in your cover letter: **In consideration of The Mount Sinai Journal of Medicine (MSJM) taking action and reviewing and editing my (our) submission, the author(s) undersigned hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership to The Mount Sinai Journal of Medicine in the event that such work is published by MSJM.** Each author must sign the statement.

Manuscript Preparation. Submit manuscripts in triplicate, including three sets of illustrations. Manuscripts must be typewritten, **double-spaced throughout (including references, legends, and tables),** on one side of 8½ × 11-in. white bond paper, with generous margins. Number pages consecutively. Begin a new page for title page, abstract, first page of text, references, each table, and legends. **Computer-generated typescripts** must be double-spaced and must be easy for editors and typesetters to read. **Computer tapes or disks:** consult managing editor.

Title Page. On the title page, on separate lines (double-spaced), include (1) title of article, phrased as concisely as possible; (2) name of each author, including first name and degree; (3) name and address of departments and institutions from which the work originated; (4) acknowledgments of sources of support; (5) name, place, and date of any conferences at which paper has been delivered; (6) the statement **Address reprint requests to . . . ,** giving full name and address, with zip code, of the appropriate author.

Abstract. Original articles should include an abstract, which should be a single paragraph not exceeding 150 words. State the purposes; basic approach or methods; main findings; principal conclusions. Emphasize new and important aspects. When an abstract is provided, a summary at the end is not necessary. Reviews and other articles without an abstract should conclude with a short summary.

Key Words. Supply 3 to 10 key words or phrases at the bottom of the title page, for use in indexing the article. Use Medical Subject Headings from *Index Medicus.*

Writing Style and Abbreviations. Authors should aim for conciseness and clarity without repetition. Avoid medical jargon, abbreviated phrasing, and obscure or newly coined abbreviations. Define all abbreviations on first use, except those for standard, universally recognized units of measurement and statistical terms. Spell out such terms as white blood cell, hematocrit, superior vena cava.

Units of Measurement. Use SI units, giving range or interval of normal values.

Editing. All papers will be edited to enhance conciseness and clarity without altering meaning and to insure conformity with journal style. The author is responsible for all statements in the article.

Illustrations. Photographs, line drawings, graphs, and charts should increase understanding of the text. Line drawings, graphs, and charts should be professionally prepared. All illustrations should be submitted in triplicate as sharp, high-contrast glossy prints. Photomicrographs must be 5⅛ in. wide. On the back of each print affix a gum label with the number of the figure (numbered consecutively in arabic numerals as cited in text), title of manuscript, name of senior author, and the word "top" with an arrow indicating top edge. Illustrations which are to appear together should be mounted accordingly as plates and should correspond to each other in size. Protect the prints by enclosing them in heavy cardboard; do not use paper clips. Photographs of patients can be published only if a copy of the permission is submitted with the photograph. Eliminate names of patients and hospital numbers from x-rays.

Legends. Legends should not duplicate the text. Double space. Number each legend to match the illustration it accompanies. Illustrations mounted as plates to appear together should be accompanied by a single legend identifying the separate elements by position ("upper left," and so on) or by letters. If letters are used in the legend, a corresponding letter must appear on the print itself. Identify all abbreviations. Indicate magnification and stain for photomicrographs.

Tables. Each table should be typed on a separate page, double spaced. Number tables consecutively as they appear in text, using arabic numerals ("Table 1"). Give each table a brief title (for example, "Effect of DMSO on Rats") and type it on a separate line. If abbreviations or symbols are used in the table, identify them in a footnote.

References. When citing references in your text, the first work cited is numbered 1, and succeeding references are numbered in sequence; enclose the citation number in parentheses and place it before any punctuation: **Cytomegalovirus (1), steroids (2), and recreational drugs (3) have all been implicated (4).** The reference list includes only works cited in the text, numbered in the order in which they are cited. Type, double spaced, following the general arrangement and punctuation style in the examples below (journal title abbreviations conform to *Index Medicus* style):

1. Nadelman RB, Wormser GP. A clinical approach to Lyme disease. Mt Sinai J Med 1990;57:144–156.

2. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. N Engl J Med 1991;324:424–428.

When a manuscript is accepted for publication, you will be asked to confirm the accuracy of all references.

Proofs. Proofs are sent to the corresponding author from the printer. Stylistic changes which do not alter meaning should not be made at this stage. Because of increased printing charges, the cost of excessive author's alterations beyond routine correction of typographical errors and major errors in data may be billed to the author. Proofs should be corrected and returned to the editorial office within 48 hours.

Reprint Orders. A reprint order form is sent to the corresponding author with proofs; return it to the editorial office.

THE
MOUNT SINAI JOURNAL
OF MEDICINE

Forthcoming:

Grand Rounds

Adrenergic Receptors in Hypertension and Heart Failure
Clive Rosendorff
Mount Sinai School of Medicine

Newer Aspects of Neuroendocrine Control in Heart Failure
Robert J. Cody
Ohio State University Medical College

Clinical and Genetic Associations in Marfan Syndrome and Related Disorders
Francesco Ramirez, Brendan Lee, and Emilia Vitale
Mount Sinai School of Medicine

Narrative and Medicine
Oliver Sacks
Albert Einstein College of Medicine

Graves' Disease
Terry Davies, MD
Mount Sinai School of Medicine

Osteoporosis: On the Verge of Rational Effective Therapy?
John Paul Bilezikian, MD
Columbia University College of Physicians and Surgeons

Theme Issues

SEPTEMBER 1992

**Human Cancer:
From Precurable to Curable**
Editors: Max Sung and Takao Ohnuma
plus Grand Rounds and general articles

NOVEMBER 1992

**Community Medicine:
In Honor of Kurt Deuschle**
Editors: Alan Silver and David Rose

JANUARY 1993

The Elderly Patient
Editors: Myron Miller and Rein Tideiksaar
plus Grand Rounds and general articles

MAY 1993

Quality Assurance
Editor: Suzanne Kramer

3 4805 0332138 3

Available now:

Toward the Conquest of Pain
Allan P. Reed, editor
volume 58, no. 3, May 1991
84 pages $15

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society
Eugene W. Straus, editor
volume 59, no. 2, March 1992
96 pages $15

New Concepts in Asthma
Zvi M. Marom, editor
volume 58, no. 6, November 1991
81 pages $15

Subscriptions for 1992: $60 individuals, U.S. and Canada; $65 all libraries; $70 individuals outside U.S. and Canada.

To order subscriptions or copies of back issues ($15 each), please send check, payable to The Mount Sinai Journal of Medicine, to the Journal at Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574; counter sales at Suite 4D, 19 E. 98th Street, New York, NY (phone: 212 241-6108; FAX: 212 722-6386).

THE MOUNT SINAI JOURNAL OF MEDICINE

OLUME 59 NUMBER 4 SEPTEMBER 1992

CONTENTS

David A. Dreiling: In Remembrance *Arthur H. Aufses Jr. and Sherman Kupfer* ... 278

GRAND ROUNDS IN CARDIOLOGY

Adrenergic Receptors in Hypertension and Heart Failure *Clive Rosendorff* ... 281

Newer Aspects of Neuroendocrine Control in Heart Failure *Robert J. Cody* ... 298

Pathophysiology and Treatment of Hypertensive Heart Disease *Robert A. Phillips* ... 309

Management of Malignant Ventricular Tachyarrhythmias *J. Anthony Gomes, Stephen L. Winters, and Arisan Ergin* ... 318

GRAND ROUNDS

Graves' Disease and Its Retroorbital Aspects *Terry Davies* ... 326

Established and New Uses of Intravenous Immunoglobulin *Charlotte Cunningham-Rundles* ... 335

ART AND SCIENCE OF CONSULTATION

The Art of the Gastrointestinal Consultation: Some Aphorisms *Henry D. Janowitz* ... 341

GENERAL ARTICLES

Clinical and Genetic Associations in Marfan Syndrome and Related Disorders *Francesco Ramirez, Brendan Lee, and Emilia Vitale* ... 350

continued inside

Veterans Affairs

Beth
Israel
Medical
Center

THE MOUNT SINAI JOURNAL OF MEDICINE

The Mount Sinai Journal of Medicine is published by The Mount Sinai Medical Center of New York and has the following affiliates: Beth Israel Medical Center, New York; Bronx Veterans Affairs Medical Center, New York; and Elmhurst Hospital Center, New York.

Editor-in-Chief
Sherman Kupfer, M.D.

Editor Emeritus
Lester R. Tuchman, M.D.

Associate Editors
Harriet S. Gilbert, M.D. Julius Wolf, M.D.

Managing Editor
Claire Sotnick

Business and Production Assistant
Claudia N. Melendez

Assistant Editors

Stephen G. Baum, M.D.
David H. Bechhofer, Ph.D.
Constanin A. Bona, M.D., Ph.D.
Edward J. Bottone, Ph.D.
Jurgen Brosius, Ph.D.
Lewis Burrows, M.D.
Joseph S. Eisenman, Ph.D.
Adrienne M. Fleckman, M.D.
Richard A. Frieden, M.D.
Steven Fruchtman, M.D.
Paul L. Gilbert, M.D.
James H. Godbold, Ph.D.
Richard S. Haber, M.D.
Noam Harpaz, M.D.
Dennis P. Healy, Ph.D.
Tomas Heimann, M.D.
Barry W. Jaffin, M.D.
Andrew S. Kaplan, D.D.S.
Samuel Kenan, M.D.
Suzanne Carter Kramer, M.Sc.
Mark G. Lebwohl, M.D.
Kenneth Lieberman, M.D.
Charles Lockwood, M.D.
Steven Markowitz, M.D.
Bernard Mehl, D.P.S.
Myron Miller, M.D.
Edward Raab, M.D.
Allan Reed, M.D.
Allan E. Rubenstein, M.D.
David B. Sachar, M.D.
Henry Sacks, M.D.
Robert Safirstein, M.D.
Ira Sanders, M.D.
Martin H. Savitz, M.D.
Clyde B. Schechter, M.D.
Michael Serby, M.D.
Phyllis Shaw, Ph.D.
George Silvay, M.D.
Barry D. Stimmel, M.D.
Nelson Stone, M.D.
Max Sung, M.D.
Carl Teplitz, M.D.
Rein Tideiksaar, Ph.D.
Richard P. Wedeen, M.D.
Michael F. Wesson, M.D.

Editorial Board

Barry Freedman, M.B.A.
Richard Gorlin, M.D.
Nathan Kase, M.D.
Panayotis G. Katsoyannis, Ph.D.
Charles K. McSherry, M.D.
Jack G. Rabinowitz, M.D.
John W. Rowe, M.D.
Alan L. Schiller, M.D.
Alan L. Silver, M.D.
Alvin S. Teirstein, M.D.
Rosalyn S. Yalow, Ph.D.

The Mount Sinai Journal of Medicine (ISSN No. 0027-2507; USPS 284-860) is published 6 times a year in January, March, May, September, October, and November in one indexed volume by the Committee on Medical Education and Publications (Owner), The Mount Sinai Hospital, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Subscription price: individuals, U.S. and Canada, $60 per year; all libraries, $65; individuals outside the U.S. and Canada, $70. Single copies, $15. New subscriptions begin with the first issue of the calendar year. Send notice of change of address 60 days before the change is effective. Second-class postage paid at New York, NY and at additional mailing offices. POSTMASTER: Send address changes to The Mount Sinai Journal of Medicine, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Copyright 1992 The Mount Sinai Hospital.

Volume 59
Number 4
September 1992

CONTENTS *continued*

Epicardial Cryoblation of Myocardial Tissue using Endocardial Thermal Isolation *Myles Edwin Lee* 357

Endometriosis Causing Colon Obstruction in Two Menopausal Women *James G. Petros, Nicholas Spirito, and Ronald Gosshein* 362

STUDENT CORNER

Ileocecal Tuberculosis in an Asian Male Immigrant: Case Report and Literature Review *Matthew R. Estill* 366

Index to Advertisers

Eli Lilly and Company page 372

Sherman Kupfer, M.D.
Editor-in-Chief
Mount Sinai Journal of Medicine
and
Arthur H. Aufses, Jr., M.D.
Chairman, Department of
Surgery
Mount Sinai School of Medicine

David A. Dreiling In Remembrance

IT IS WITH DEEP REGRET that the *Journal* notes the death of Dr. David A. Dreiling on September 24, 1991, at the age of 73. Dr. Dreiling had served as the associate editor and then editor-in-chief of the *Journal* since 1960, about half of his life. What was so remarkable about this service to the *Journal* over this long span, in addition to its quality and creativity, was his commitment to so many other activities here at Mount Sinai and the biomedical community throughout the world. He epitomized the oft-quoted expression, "if a job has to be done, give it to a busy man." It has been said that David spent his entire professional life at Mount Sinai, and we guess that is technically correct. In reality, he had an overwhelming professional presence in many of the world's academic health centers.

David was born in Brooklyn, and graduated summa cum laude from Boys High School. He attended Cornell University for his undergraduate work, and was elected to membership in Phi Beta Kappa. He graduated summa cum laude. At New York University School of Medicine he was elected to Alpha Omega Alpha, the honorary medical fraternity. Following his internship and surgical training at The Mount Sinai Hospital, he became involved in research in pancreatic physiology and inflammatory diseases of the pancreas. He was recognized to be one of the world's leading scientists in this field. He also served as director of the Experimental Gastrointestinal Surgery Laboratory, director of the Animal Research Facility, and chief of surgery at Greenpoint Hospital and then at the City Hospital Center at Elmhurst, which had become affiliated with Mount Sinai. He served as the first Florence and Alfred E. Gross Professor of Surgery and acting chairman of the Department of Surgery at the Mount Sinai School of Medicine, and for 15 years as vice chairman of that department. He was awarded the Jacobi Medal for service to Mount Sinai.

David was an astute clinician, a master surgeon, and an inspiring teacher at all levels. He had a unique ability to stimulate young investigators, and they flocked to him from far and wide. He was a fellow of the American Surgical Association, and served as president of the American College of Gastroenterology, the first surgeon ever to hold that position.

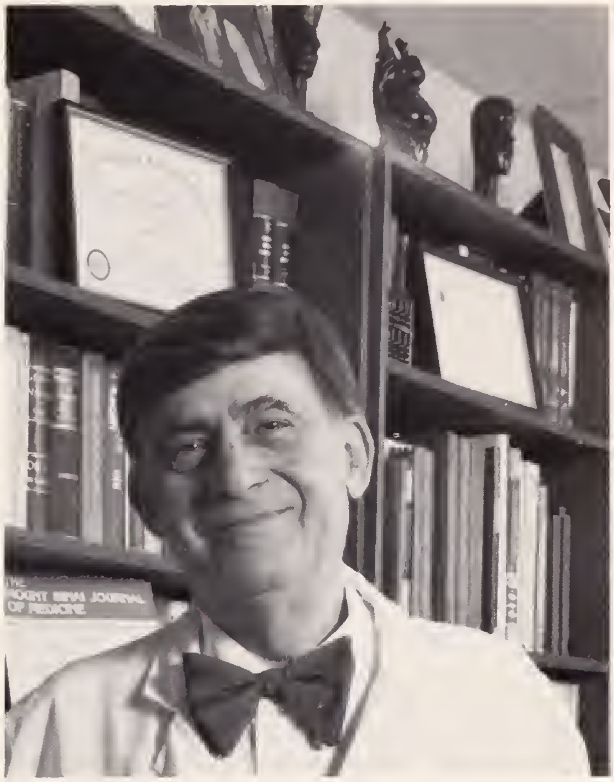
He was no xenophobe. He was the founder of the International Society of Pancreatology and was a member of some 60 national and international organizations. His trainees hold positions of renown throughout the world in the field of gastroenterology and gastrointestinal surgery and research. He maintained contact with all of his trainees throughout his professional life and enjoyed every minute of it. He truly was a citizen of the world. He was invited to present scientific papers in almost every corner of this earth. He was a fellow of societies of gastroenterology and medicine in Venezuela, Chile, Peru, and Portugal, and the Royal Society of Medicine of England. His hospitality was legion; his home clearly served as "Hotel Dreiling" to so many visitors from here and abroad.

David was an inveterate storyteller, a historian, and a loyal, devoted Saturday night Metropolitan Opera goer and lover beyond the expected. At intermission, he was easy to spot at the bottom of the Grand Staircase, where he delighted in discussing the qualities of the performance. He was involved in the arts, music, literature, antiques, Orientalia, good food, great wine, and orchids of every variety. His home was resplendent of each of these involvements.

If all of this was not enough, David served as editor-in-chief of the *American Journal of Gastroenterology,* art editor of *G.I. Tract,* Secretary of the Biomedical Engineering Section of the New York Academy of Medicine, and too many other activities to be mentioned in the available space. There is one omission from his curriculum vitae which is worthy of mention, though. He served for many years as a member of Mount Sinai's Research Committee and the Institutional Review Board. When David was present at these meetings, which was almost always, it was a fact that the agenda had to be completed in no more than two hours, since he could be counted on to make a motion to adjourn at that point.

All who knew David were aware of his distinctive, perhaps even outrageous bow ties, tartan sport jackets, fur coat or cape, and hats. He was a family man's family man, with an enormous love for his devoted wife Muriel, his son and daughter-in-law David Jr. and Ann, and the grandchildren, Jesse, Sam, and Katie.

His presence is sorely missed.



David A. Dreiling

The David A. Dreiling Award For Academic Achievement, Department of Surgery, Mount Sinai School of Medicine

The David A. Dreiling Award for Academic Achievement has been established within the Department of Surgery. This award honors that graduating chief resident who demonstrates overall excellence in scholarly endeavors. The first awardee, in 1991, was Dr. Edward Shlasko, and Dr. Sander Binderow is the 1992 recipient.

Adrenergic Receptors in Hypertension and Heart Failure

Clive Rosendorff, M.D., Ph.D.

The person for whom this lecture was named, Dr. Alexander B. Gutman, was head of the Department of Medicine at The Mount Sinai Hospital for many years and was one of the most distinguished physicians among many who have graced this institution. Several of his editorials in the *American Journal of Medicine,* of which he was the founder and editor for many years, emphasized the mutually enriching interaction between clinical medicine and the basic biomedical sciences, a theme which has been the basis of my professional life.

The first measurement of arterial blood pressure was made about 1731 by the Reverend Stephen Hales (1), who inserted a glass tube into the crural artery of a horse and measured the height to which the column of blood rose (Fig. 1). In a sense, Hales was one of my academic predecessors, but my personal link with him is confined to my having lived with my family for about six years in Teddington, in Middlesex, England, not far from his vicarage as parish priest.

My discussion here is about the autonomic nervous system, particularly the sympathetic nervous system, in hypertension and in congestive heart failure. Under the rubric of hypertension I deal briefly with central autonomic mechanisms. I do not talk much about plasma catecholamines, because I think the burden of the literature is that the majority of patients with essential hypertension have elevated plasma catecholamine levels (2)—and that is about all that is known on that subject. I do discuss at some fair length neurotransmitter processes, particularly in relation to adrenergic receptors, and then cover the more recent and perhaps more exciting area of the cascade of events which couple receptor activation with the contractile proteins in vascular smooth muscle cells.

The Autonomic Nervous System in Hypertension

Central Pathways. Fig. 2 shows the proposed pathways and neurotransmitters involved in the central nervous system control of blood pressure, including the baroreceptor reflex pathway (3, 4). Afferents from the ninth and tenth cranial nerves synapse in the nucleus of the tractus solitarius, which connects with the ventrolateral medulla, then to the intermediolateral column of the spinal cord, thence to the classical preganglionic fibers, terminating in the sympathetic ganglia and in the adrenal medulla and the classical postganglionic fibers that innervate the myocardium and the vasculature.

The point to note is the great diversity of putative neurohumoral transmitters, including L-glutamate, substance P, norepinephrine, epinephrine, GABA, neuropeptide Y, and of course, acetylcholine at the preganglionic nerve terminals, and the transmitters released by postganglionic neurons, including norepinephrine, dopamine, and serotonin. Little is known about the control of these neurotransmitters, and not too

---

Adapted from the Alexander G. Gutman Memorial Lecture for 1990 given by the author on December 18, 1990 at the Mount Sinai School of Medicine, revised and updated May 1992. From the Departments of Physiology and Medicine, University of the Witwatersrand Medical School, Johannesburg, South Africa. Address reprint requests to the author, now Professor and Associate Chairman, Department of Medicine, Mount Sinai School of Medicine, New York, NY, Chief, Medical Service, Veterans Affairs Medical Center, Bronx, NY, and Director, The Mount Sinai Hypertension Program, at Box 1118, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029.

Fig. 1. The Rev. Stephen Hales measuring blood pressure of a horse. Like most directors of research, he is sitting (with a glass of ale) while his assistant does the work. *Reprinted* from *Medical Times,* 1944.

much work has been done on the modification of these transmitter processes as therapeutic tools. There is clearly an enormous potential for designing drugs that will in some way modify or modulate these reflexes.

Adrenergic Receptors in Vascular Smooth Muscle. It has been known for a long time that sympathetic neurohumoral transmission consists basically of the release of the major α_1-agonist norepinephrine from sympathetic nerve terminals to activate postjunctional α_1 adrenoceptors, and that α_2 receptors, on the sympathetic nerve terminal itself, will mediate feedback inhibition of norepinephrine release. The sort of experiment which illustrates this point, previously reported in the dog (5, 6) and in my laboratory in the baboon, is shown in Fig. 3. Segments of baboon mesenteric artery, with innervation intact, were subjected to electrical stimulation via the nerve supply, and the release of pre-incubated ^{3}H-norepinephrine (^{3}H-NE) and the contraction of the artery were measured. Prazosin, an α_1 antagonist, had no effect on the release of NE but reduced the contractile response. The α_2 antagonist yohimbine, by blocking prejunctional α_2 receptors, allowed for the uninhibited release of ^{3}H-NE from the preparation and consequently enhanced contractility. Phentalomine, a nonselective α_1 and α_2 antagonist, enhanced norepinephrine release (α_2 blockade) and reduced contractility (α_1 blockade). All of these actions are perfectly consistent with the idea that α_1 receptors are postjunctional and α_2 receptors prejunctional, and that α_2 receptors inhibit the norepinephrine release from sympathetic nerve terminals.

The same is true in the heart, but in that organ the major adrenergic receptor is a β_1 receptor, which can of course be blocked with selective β_1 antagonists. Stimulating the cardiac sympathetic nerves at various frequencies and measuring the heart rate response (Fig. 4) shows that the α_1-selective blocker, prazosin, has no effect, with a curve virtually identical with control, whereas phentolamine, which blocks both α_1 and α_2 receptors, causes an enhancement of the response because of the blockade of the prejunctional α_2 modulation of norepinephrine release.

In the past ten years also, analysis of the subtypes of α and β receptors has become much more sophisticated and the subtypes have proliferated (Table). Receptors for α_1 and α_2 can each be subdivided into α_{1A} or α_{1B}, or α_{2A} α_{2B}, or α_{2C4}, on the basis largely of the potency order of the agonists or of the selectivity of the agonists and antagonists. Similarly, β receptors may be subdivided into β_1 and β_2. Some of the definitions then be-

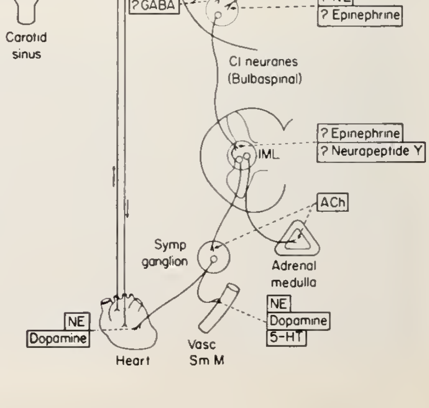
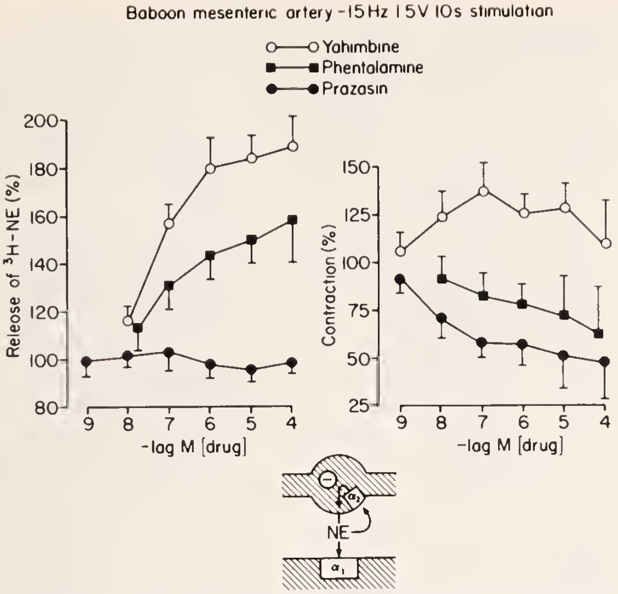


Fig. 2. Proposed pathways and transmitters involved in central nervous system control of blood pressure, including baroreceptor reflex pathway. Afferents from carotid sinus and heart via cranial nerves IX and X terminate in nucleus tractus solitarius (NTS), with, possibly, L-glutamate and substance P as neurotransmitters. Neurons of NTS relay into C_1 area and may release γ-aminobutyric acid (GABA) at tenth nerve nucleus and ventrolateral medulla, where they synapse with C_1 neurons of bulbospinal tract. Also terminating in ventrolateral medulla are descending fibers from hypothalamus, which are adrenergic. C_1 neurons relay to intermediolateral column of spinal cord and release either epinephrine or neuropeptide Y. Preganglionic fibers of peripheral autonomic efferent originate in intermediolateral column and terminate in adrenal medulla or sympathetic ganglia, releasing acetylcholine (ACh). Postganglionic fibers to heart and vascular smooth muscle release norepinephrine (NE), although these sites also have dopamine and serotonin (5-HT) receptors. *Adapted* from Reis et al. (3).



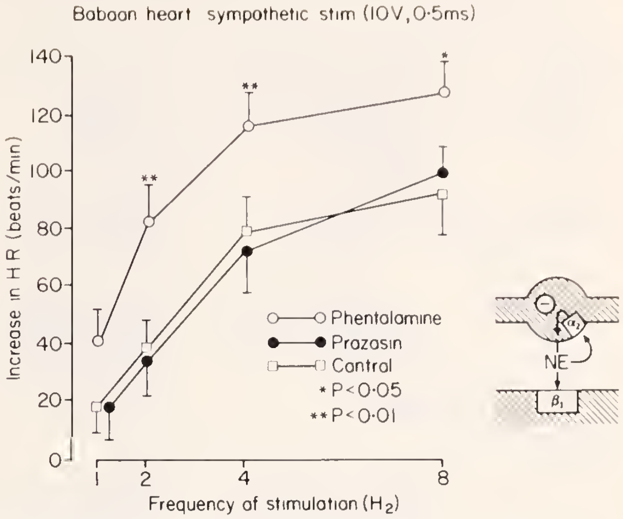


Fig. 3. Left Stimulation of sympathetic nerve supply to an in situ segment of baboon mesenteric artery. **Fig. 4.** Right Stimulation of cervical sympathetic nerves and ganglia effect on heart rate in baboon.

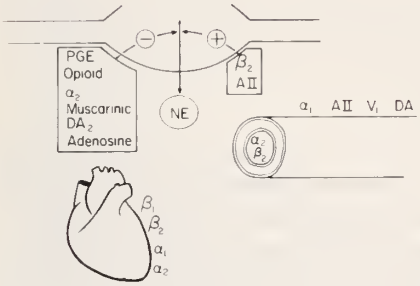

PGE
Opioid
α_2
Muscarinic
DA$_2$
Adenosine
NE
β_2
AII
α_1 AII V$_1$ DA
α_2
β_2
β_1
β_2
α_1
α_2

Fig. 5. Prejunctional modulation of norepinephrine (NE) release, and distribution of adrenoreceptors in myocardium and arteries. PGE, prostaglandins of E series; DA, dopamine; AII, angiotensin II; V, vasopressin.

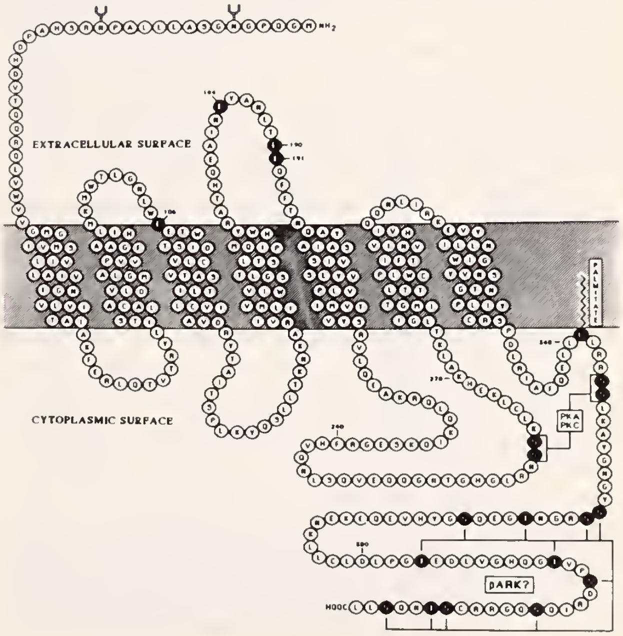

NH$_2$
EXTRACELLULAR SURFACE
CYTOPLASMIC SURFACE
PALMITATE
PKA
PKC
βARK?
HOOC

Fig. 6. Proposed arrangement of human β_2-adrenergic receptor within cell membrane. (circle, an amino acid): 7 transmembrane hydrophobic helices, 3 intracytoplasmic loops, and 3 extracellular loops. Also shown are sites of posttranslational modification. There are two sites of extracellular N-linked glycosylation, four cysteine residues that may participate in disulfide bonds, an extracellular cysteine residue that may serve as site of attachment for a palmitate membrane anchor, as well as multiple intracellular threonine and serine residues, potential sites of phosphorylation by protein kinase A (PKA), protein kinase C (PKC), or β-adrenergic receptor kinase (βARK). *Reprinted with permission* from Raymond et al. (9).

TABLE

Classification of Adrenergic Receptors

	α_1	α_2	β_1	β_2
Agonist	NE ≥ EPI > PE > ISO	EPI ≥ NE > PE > ISO	ISO > NE ≥ EPI > PE	ISO > EPI > NE > PE
Selective agonists	PE, Cirazoline Methoxamine	Clonidine BHT 920 UK14304	Xamoterol Denopamine	Procaterol Zinterol
Selective antagonists	PRAZ Corynanthine	Yohimbine Rauwolscine Idazoxan	CGP 20712A Betaxolol Atenolol Metoprolol	ICI118551, 118537 Butoxamine
Radioligands	[^{3}H]Prazosin [^{125}I]HEAT (BE2254)	[^{3}H]Rauwolscine [^{3}H]Yohimbine [^{3}H]UK14304	[^{3}H]Bisoprolol	[^{3}H]ICI11581
Effector pathways	IP_3/DG	cAMP ↓	cAMP ↑	cAMP ↑
Subtypes	α_{1A} WB4101 > PRAZ couples to dihydropyridine-sensitive Ca^{2+}CH α_{1B} PRAZ > WB4101	α_{2A} Oxymetazoline > PRAZ (250 nm) ? couples to PIP_2 hydrolysis α_{2B} Oxymetaxoline = PRAZ (5 nM)		

EPI, epinephrine; NE, norepinephrine; PE, phenylephrine; ISO, isoproterenol; PRAZ, prazosin; Ca^{2+}CH, calcium channel; PIP_2, phosphatidyl-inositol-bisphosphate; HEAT, 2-β-4-hydroxy-3-iodophenylethylaminomethyltetralone; IP_3, inositol-trisphosphate; DG, diacylglycerol.

come somewhat complex and occasionally circular, but the classification has been useful and functional (7–9).

Prejunctional α_2 receptors cause an inhibition of norepinephrine release, but we now know that a family of such receptors is located on the sympathetic nerve terminals, and that these receptors bind and respond to a number of agonists (Fig. 5). These include receptors for prostaglandins of the E series, opiates, acetylcholine, dopamine, and adenosine. We now also have a family of prejunctional receptors which enhance norepinephrine release. These are β_2 and angiotensin II receptors. I think it is generally accepted that α_1, angiotensin II, vasopressin, and dopamine receptors are situated in vascular smooth muscle at the junction of the outer media and the adventitia where the innervation density is greatest, and therefore probably respond primarily to released neurotransmitter from sympathetic nerve terminals. By contrast, α_2 and β_2 receptors have their highest density at the media-interna junction, where sympathetic nerves are sparse, and therefore probably respond to circulating catecholamines.

In the heart are families of receptors of which the most important is β_1, but also including β_2, α_1, α_2 and probably dopamine and angiotensin II receptors as well. In smooth muscle cells there are also purinergic receptors, which respond to ATP coreleased from sympathetic nerve terminals, and which generate extrajunctional potentials and probably the phasic contractile phase of the vascular smooth muscle, in contrast to the α_1 activation which may primarily mediate tonic contraction (10).

More recently there has been an explosion in knowledge of the structure and the structure-function relationships of adrenergic receptors. Using the methods of molecular biology, Lefkowitz and his colleagues (9) have shown that there is a considerable degree of structural homology between adrenergic receptor subtypes; they all contain seven highly hydrophobic domains which are probably the segments of the receptor which span the membrane (Fig. 6). The amino end of the receptor protein is extracellular and subserves the binding of ligands (agonists and antagonists) to the molecule, whereas the carboxyl end is intracellular and is probably the component which activates the cascade of the excitation-contraction coupling process.

With my colleagues U'Prichard and Hurwitz, I was privileged quite a long time ago to be able to demonstrate, I think for the first time using the techniques of radioligand binding, that there are adrenergic receptors in vascular smooth muscle (11). Before that, all the evidence had been indirect and based on classical pharmacologic techniques. We demonstrated saturable binding of α_1-specific ligands (^{3}H-WB4101 and ^{3}H-prazosin) with linear Scatchard plots and Hill numbers around one, indicating the presence of α_1 noradrenergic receptors in the tunica media of large and intermediate sized arteries. Similarly, ^{3}H-clonidine and ^{3}H-p-aminoclonidine binding and displacement studies confirmed the presence of α_2 receptors. This was also the first direct demonstration by ligand binding that vascular smooth muscle contains both α_1 and α_2 receptors.

Since then our techniques have become much more sophisticated. Fig. 7 shows the binding of ^{3}H-dihydroalprenolol, a β-receptor antagonist, to baboon red blood cell ghosts, showing specific binding obtained by subtracting the quantity bound nonspecifically from the total binding (12). The insert, the Scatchard plot, shows that B_{max} (the intercept on the x axis), a measure of the density of binding sites, is a little over 300 fmols/mg. The slope of that line is an index of the affinity of the ligand with the binding site.

Adrenergic Receptors in Hypertension. Using these techniques, we began to investigate what happens to adrenergic receptors in systemic hypertension. In order to do this we had to evolve an animal model of hypertension, and were fortunate in South Africa of having a number of non-human primates, including baboons, which are available relatively inexpensively. We settled on the Grollman two-kidney, two figure-of-eight model of hypertension in the baboon (13). The technique involved tying a tape around the kidney in a figure-of-eight configuration, and it is presumably the local areas of focal cortical ischemia which give rise to the hypertension. Once the two-kidney, two figure-of-eight renal state is induced, blood pressure goes up quite briskly, and at 24 weeks the mean arterial blood pressure is about 150 mmHg.

The main findings are summarised in Fig. 8. There was a striking increase in the concentration of α_1- and α_2-receptors in arteries, lung, and kidney and a large decrease in β-receptor density in the myocardium, lung, and kidney (14). At that time we were unable for technical reasons to measure the β-receptor density in vascular smooth muscle. Generally the striking and consistent finding in a variety of animal models is that there is upregulation of α_1 and α_2 receptors and downregulation of β-receptors.

Finding changes in the density of receptors is not particularly interesting unless one can also find some sort of functional correlate of these changes. Fig. 9 shows the dose-response curve of phenylephrine versus the increase in arterial pressure as an index of postjunctional α_1 and α_2

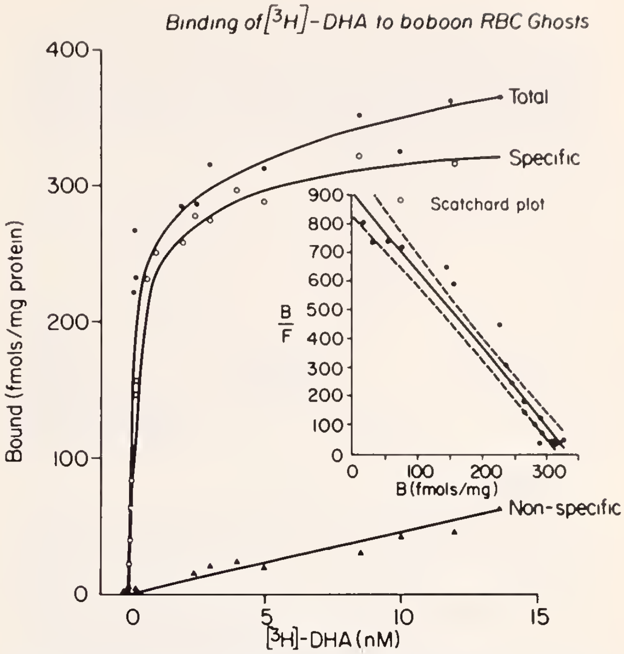


Fig. 7. Saturation isotherm of total, nonspecific, and specific binding of ^{3}H-dihydrolaprenolol to baboon erythrocyte ghosts. Inset: Scatchard plot of specifically bound ^{3}H-DHA. *Adapted* from Susanni et al. (12), and reprinted with permission.

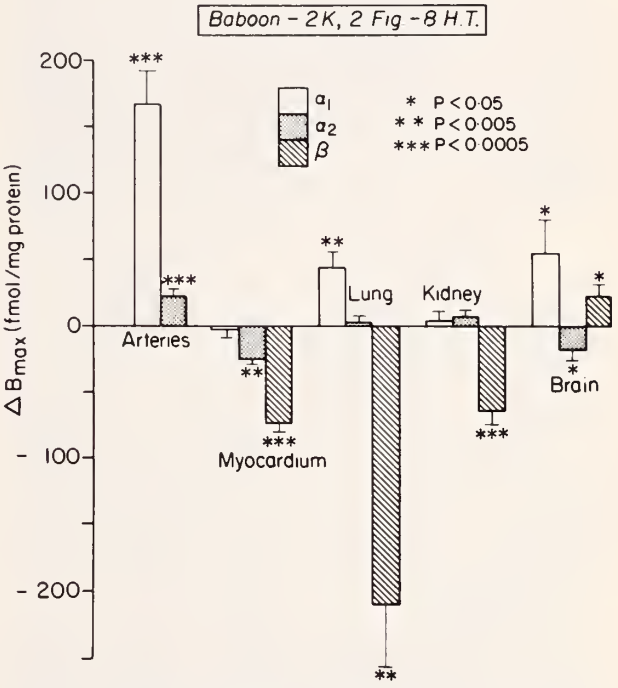


Fig. 8. Percentage difference in density of α_1, α_2, and β receptor (Δ B_{max} %) in arteries, myocardium, lung, kidney, and brain of baboons with two-kidney, two figure-of-eight hypertension, compared with shamoperated controls. *Reprinted* from 14, and unpublished data.

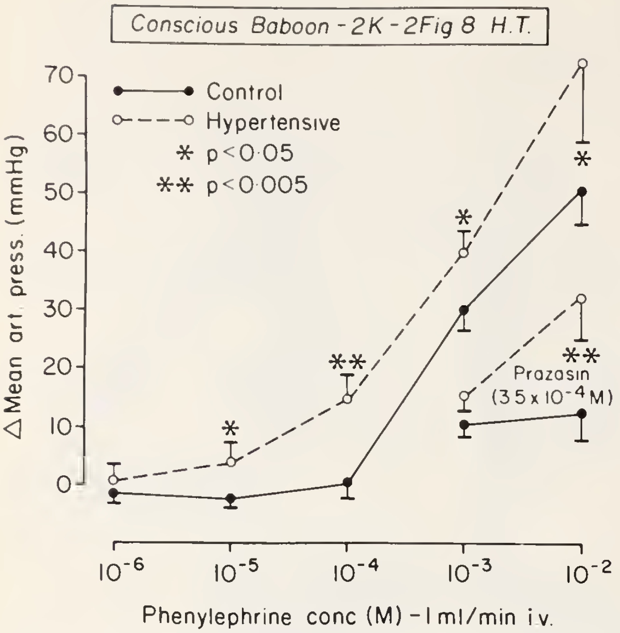


Fig. 9. Dose-response curve of phenylephrine, an α_1-receptor agonist, versus change in aortic mean blood pressure from baseline. Means ± SEM.

receptor activity in this particular model. Hypertensive animals show a leftward shift of the dose-response curve, that is to say, a sensitization to the effects of phenylephrine, completely consistent with the upregulation of α_1 receptors that we found by means of the ligand-binding techniques.

Similar results have been found in humans. In patients with hypertension, Weidman et al. (15) showed that the norepinephrine pressor dose (the dose of norepinephrine required to increase the mean arterial blood pressure by a specified amount) is lower in patients with essential hypertension than it is in normal subjects, indicating a supersensitivity to norepinephrine in these patients. These changes have also been shown in the case of the β-receptor responses. If there is downregulation of cardiac β receptors, as we found in the baboon model of hypertension (14), one would expect that the dose response curve of a β agonist versus heart rate would be shifted rightwards, and that is indeed the case (Fig. 10). In humans, Bertel et al. (16) showed that the dose of isoproterenol required to increase the heart rate by 25 beats per minute is higher in hypertensive patients than in normotensive controls, which is consistent with downregulation of β receptors.

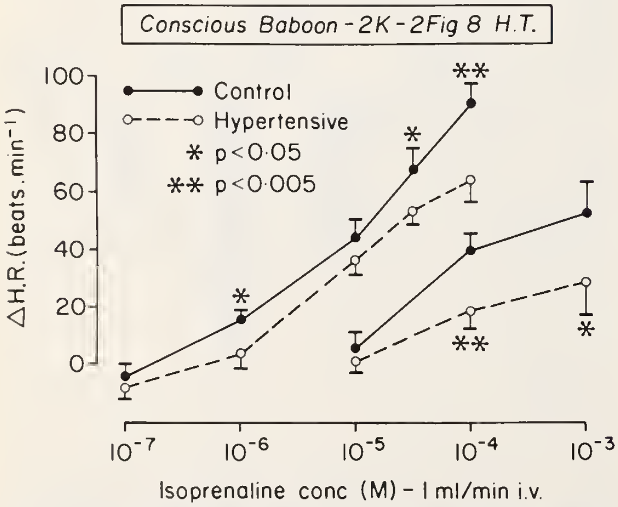


Fig. 10. Dose-response curve for isoproterenol, a nonselective β agonist, versus change in heart rate from baseline. Means ± SEM.

The question then arose: are these changes of α- and β-receptor density a consequence of the hypertension, or could they in some way be implicated as part of the pathophysiologic process which causes the hypertension? It seemed that, to answer this question, the temporal sequence of these events was important. If it could be shown that some of these changes preceded the onset of the hypertension, it was clear that such changes could not be the consequence of the hypertension but were involved in the pathogenesis. The answer came from a study by Michel et al. (17) in which it was shown that lymphocyte β_2-adrenoreceptor density (assessed by ^{125}I-iodocyanopindolol binding) and responsiveness (assessed as isoproterenol-induced cAMP increases) did not differ between normotensive children of normotensive parents and normotensive children of hypertensive parents. However, platelet α_2-adrenoceptor density (assessed by ^{3}H-yohimbine binding) was significantly higher in children with one essential hypertensive parent. We can conclude that platelet α_2- but not lymphocyte β_2-adrenoceptor changes in essential hypertension are genetically determined, and the α_2 upregulation may be important in the pathogenesis of the hypertension while the β_2-adrenoceptor downregulation is probably a consequence of hypertension.

Signal Transduction. Some important downstream events are concerned with signal transduction or the continuity of information transfer between the receptors and the contractile proteins (Fig. 11). After binding with an agonist, the α_1 receptor, though a guanine nucleotide regulatory protein (GNRP), will activate phospholipase C, the result of which is the formation, from phosphatidyl inositol bisphosphate (PIP_2), of the second messengers inosotol trisphosphate (IP_3) and 1,2-diacylglycerol. IP_3 will bind with an IP_3 receptor on the endoplasmic reticulum and cause the release of Ca^{2+} through Ca^{2+}-release channels into the cytoplasm. Diacylglycerol will stimulate protein kinase C, which has several important actions including the activation of the Na^+-H^+ exchanger with the consequent increase in intracellular pH and the stimulation of growth and proliferation.

β and α_2 Receptors have a different mechanism of action. Both work through adenylate cyclase, stimulated by β receptors via a stimulatory G protein (G_s) and inhibited by α_2 receptors via an inhibitory G protein (G_i). Adenylate cyclase catalyses the bioconversion of ATP to cAMP, which in turn activates protein kinase A, which phosphorylates and therefore activates myosin light chain kinase (MLCK).

Calcium binds with calmodulin to activate myosin light chain kinase, which is the key substance in the phosphorylation and therefore activation of myosin to cause contraction.

It is in this area that all of the excitement is occurring now; the signal transduction mechanisms are highly complex and are being intensively researched, and there is some evidence that these are abnormal at least in one animal model of hypertension. The accumulation of inositol phosphate was significantly greater in arteries from DOCA hypertensive rats than from normotensive rats both in whole tissue (18) and in norepinephrine-stimulated primary cell cultures (19). A number of other steps in the excitation-contraction coupling sequence are also abnormal in hypertensive animals and in patients who have essential hypertension. Not all of the results, however, have been positive. We completed some studies recently (20) in which we looked for differences between spontaneously hypertensive rats (SHR) and control normotensive Wistar Kyoto rats (WKR) in respect of angiotensin II stimulation of primary aortic smooth muscle cell cultures. There was no difference between the two strains with respect to either basal or angiotensin II stimulated myosin light chain phosphorylation.

Interrelationships between Receptors. There are some very important interrelationships between the adrenergic receptors and other signal transduction systems. For example, we don't really know how adrenoreceptors on vascular smooth muscle cell membranes open Ca^{2+} channels, and what the interactions with angiotensin II receptors are. In a study on patients with mild to moderate essential hypertension (21) we constructed norepinephrine and angiotensin II versus diastolic and systolic blood pressure dose response curves (Fig. 12). In patients with hypertension treated with therapeutic doses of nitrendipine, a dihydropyridine Ca^{2+} channel antagonist, the blood pressure responses to both of these pressor agents decreased. This suggested that in these patients some of the action of the norepinephrine and the angiotensin II is mediated via dihydropyridine sensitive Ca^{2+} channels. These have been characterised as L-type voltage-operated Ca^{2+} channels (22).

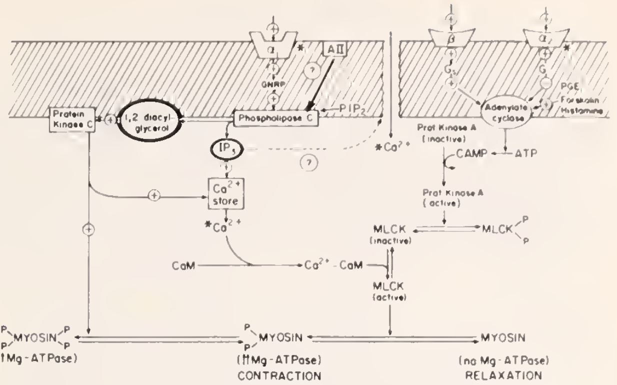


Fig. 11. Adrenergic receptor-contraction coupling in vascular smooth muscle.

Cellular and Molecular Biology. In the history of any research field there sometime occurs a paradigm shift, bringing with it new insights and a totally original orientation. I believe this has happened in vascular biology, where the techniques of classical pharmacology have largely been superseded by those of cellular and molecular biology. With this development has come the

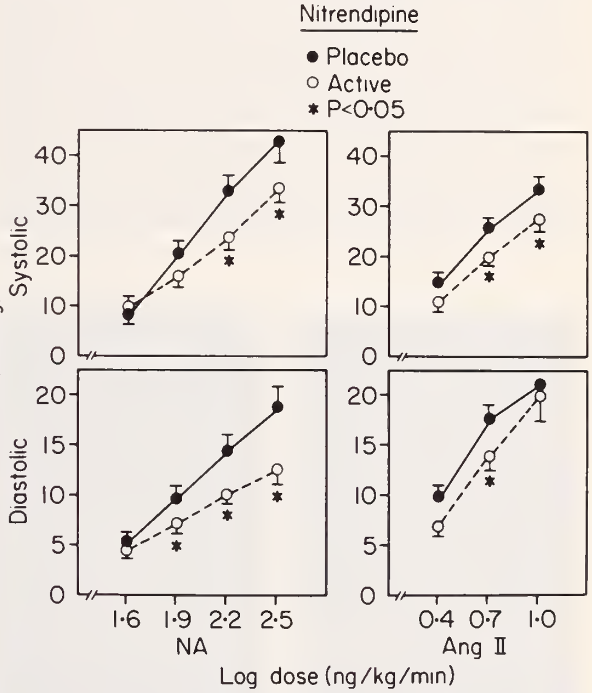


Fig. 12. Log dose versus steady state increases in systolic and diastolic blood pressure, for norepinephrine (NA) and angiotensin II (Ang II) IV infusions. Means ± SEM. Levels of statistical significance are between mean responses to same dose of NA or Ang II for placebo vs active groups. *Reprinted* with permission from Rosendorff et al. (21).

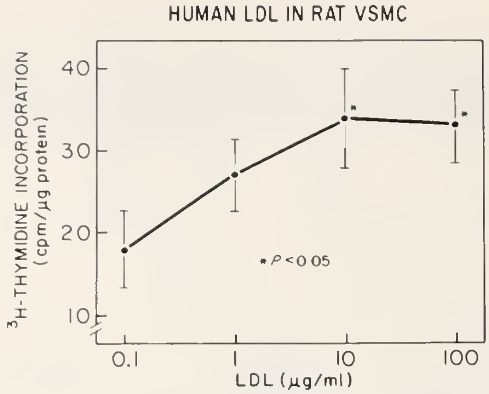


Fig. 13. Incorporation of tritiated thymidine into rat vascular smooth muscle cells at four different concentrations of low-density lipoprotein added to medium (0.5% calf serum) 8 hr before incubating cells with 2 μCi of ^{3}H-thymidine for 6 hr.

realization that there has been a tremendous overlap between vasoactive and mitogenic agents, between those agents which activate or inhibit contractile processes and those which stimulate cell growth and/or proliferation. For example, endothelin, a highly potent vasoconstrictor, stimulates proliferation of Swiss 3T3 fibroblasts (23, 24) and vascular smooth muscle cells (25, 26). We are currently studying the mechanism of action of similar effects of a variety of adrenergic agonists and angiotensin II.

LDL as Mitogen. We have also been interested in low-density lipoprotein (LDL) cholesterol from a slightly unusual point of view. Most researchers are concerned with the cholesterol deposited in atheromatous vascular lesions, but we have for many years been studying cholesterol as a vasoconstrictor, or more precisely as potentiator of norepinephrine-induced vasoconstriction (27, 28). The mechanism is primarily an upregulation of α_1 and α_2 adrenoceptors in vascular smooth muscle (29). There is also an increase in B_{max} for β receptors in the myocardium (30). LDL also stimulates vascular smooth muscle proliferation. ^{3}H-thymidine incorporation into cultured rat vascular smooth muscle cells is increased in a dose-dependent fashion by the addition of human LDL to the culture medium (Fig. 13). Human LDL also causes a rapid transient increase in cytosolic Ca^{2+} concentration, and alkalinization. The alkalinization does not occur in a Na^+-free medium, suggesting that the alkalinization is dependent on activation of Na^+-H^+ exchange (31). These findings are compatible with the idea that, at least in this model, Ca^{2+} mobilization and intracellular alkalinization may accompany cell proliferation in response to LDL.

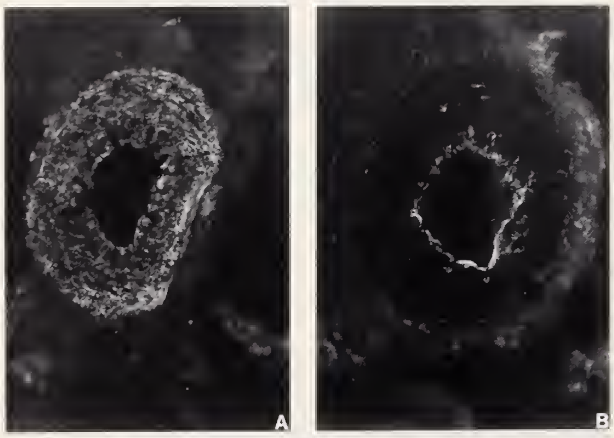


Fig. 14. Immunolabelling of c-fos and c-myc proto-oncogene products in rat aorta. Sections were incubated with polyclonal anti-c-fos (A) and anti-c-myc (B) antibodies.

So LDL cholesterol is not only passively deposited in atherosclerotic lesions, but clearly also sensitizes vascular smooth muscle α_1 agonists by upregulating the α_1 receptor, and also stimulates vascular smooth muscle proliferation, possibly by calcium, and possibly by the process which involves Na^+-H^+ exchange. Therefore, we should heed the biblical injunction, “Eat neither fat nor blood” (Leviticus 3: 17 and 7: 23).

Proto-oncogenes. Lastly, under the rubric of hypertension it should at least be mentioned that there has been an explosive increase in research interest in the growth and proliferation of vascular smooth muscle cells and proto-oncogenes. Some proto-oncogene products, like c-sis (platelet derived growth factor-B; PDGF-B) and k-fgf/hst (fibroblast growth factor; FGF), are themselves growth factors; some, like c-erb, make receptor protein; and some, like c-fos, c-myc, and c-jun, are probably early growth-regulating nuclear proto-oncogenes (32). Fig. 14 shows an immunohistochemical study, done by Hanna Benze and me in Lydie Rappaport’s laboratory in Paris, showing c-fos in the media of rat aorta and c-myc located primarily in the intimal cells. The growth-regulating proto-oncogenes are inducible; cell growth, differentiation, and development can be initiated by a wide variety of agents (32, 33), including angiotensin II (34, 35).

The potential of all of these findings for devising new strategies for treating the vascular hyperplasia and hypertrophy of hypertension is enormous. Obvious candidates are angiotensin-converting enzyme (ACE) inhibitors (36–38) and angiotensin II antagonists. An example: a study by Christensen et al. (38) in which he measured the media:lumen ratio in spontaneously hypertensive rats (SHR), in the control Wistar-Kyoto

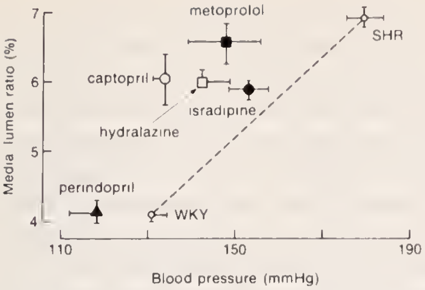


Fig. 15. Decrease in media:lumen ratio in spontaneously hypertensive rats (SHR) treated for 20 weeks with metoprolol (130 mg/kg/day), isradipine (42 mg/kg/day), hydralazine (25 mg/kg/day), captopril (60 mg/kg/day), and perindopril (1.5 mg/kg/day). All drugs except metoprolol reduce media:lumen ratio, peridopril somewhat more than the others. Doses used were clearly not equipotent for blood pressure reduction; therefore, differences in media:lumen ratio between perindopril and the others must be treated with reserve. *Redrawn, and reprinted* with permission from Christensen et al. (38).

strain of rats, and in SHR treated with a variety of antihypertensive drugs (Fig. 15). Media:lumen ratio was significantly decreased after treatment with the ACE inhibitors captopril and perindopril, the Ca^{2+}-channel blocker isradipine, and hydralazine.

Summary. There are complex central autonomic pathways subserving cardiovascular reflexes and control with a variety of neurohumoral transmitters. Plasma catecholamines are generally increased in hypertension. Receptors are mainly of the α and β subtypes, determined by the potency order and selectivity of their agonists and antagonists. The α_1 receptors are primarily postjunctional, and the α_2 receptors are both prejunctional and extrajunctional. The β_2 receptors found in vascular smooth muscle and are extrajunctional. The essential changes in hypertension are those of upregulation of the α receptors and downregulation of β receptors. The α receptor changes precede the onset of the hypertension, and therefore may be causal, while the β receptor changes do not precede the onset and may be some sort of consequence or regulatory response to the elevated blood pressure.

Signal transduction involves activation of G proteins, which affect the rate of synthesis of second messengers, IP_3, DAG, and cAMP. These are altered in hypertension. Vascular cell growth and proliferation is stimulated by adrenergic agents, and many other growth factors or mitogens, including LDL and angiotensin II, acting in many cases by inducing the proto-oncogenes c-fos, c-jun, and c-myc. Some antihypertensive drugs, especially ACE inhibitors and Ca^{2+} channel blockers, can prevent or reverse this process.

The Autonomic Nervous System in Congestive Heart Failure

Sympathetic Activity Increases in Heart Failure and Makes It Worse. The sympathetic nervous system is activated in patients with heart failure. In these patients, urinary excretion of catecholamines and plasma norepinephrine concentration are considerably higher than in normal subjects (39–41). Studies using tritiated norepinephrine have indicated that these elevated levels are due not only to alterations in norepinephrine metabolism but also to increases in synaptic cleft concentrations, the result of increased sympathetic nerve activity. It has also been shown that the prognosis is considerably impaired in patients who have a plasma circulating norepinephrine concentration greater than 600 pg/mL (41). So there is no question that circulating catecholamines are an index of an overactive sympathetic nervous system and a poorer prognosis. Why is this so? There can be a number of reasons (Fig. 16) why increased sympathetic tone is deleterious in the patient with congestive heart failure (42). The activation of the renin-angiotensin-aldosterone system by sympathetic neurones activating β-adrenergic receptors in the kidney may result in an increased preload and may, because of the growth-stimulating action of angiotensin II, cause myocardial hypertrophy. Circulating norepinephrine and angiotensin II will increase the left ventricular afterload and therefore increase LV myocardial wall stress. There is probably direct myotoxicity of overactive sympathetic nerves, and their arrhythmogenic effect is well known.

Cardiac Autonomic Receptors in Heart Failure. The arrangement of the receptors in cardiac myocytes and the downstream transduction mechanisms are similar to those in vascular smooth muscle, with a few important differences. One is that there are important muscarinic receptors which respond to acetylcholine, to activate the GTP inhibitory proteins (G_i) which couples the muscarinic receptor to adenylate cyclase (43), to increase the efflux of K^+ from cells, resulting in hyperpolarization of the cell membrane and to decreased Ca^{2+} movement into cells through the L-type dihydropyridine-sensitive Ca^{2+} channels (44).

Another difference is that the major adrenergic receptor is a β_1 receptor. Quite a long time ago

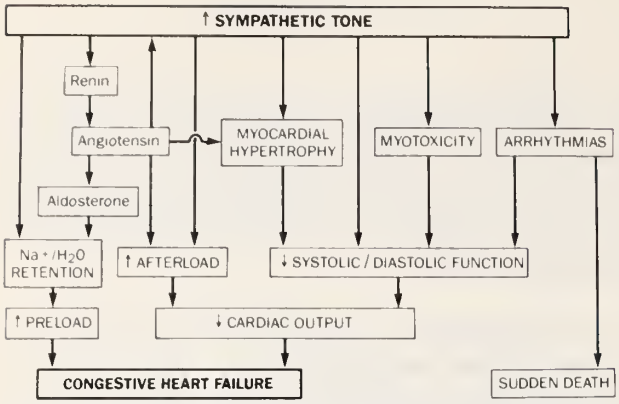


Fig. 16. Schematic diagram of possible adverse effects of increased sympathetic tone in congestive heart failure. *Adapted* with permission from Daly and Sole (42).

we were able to show that in left ventricular failure, the density of ventricular β adrenoreceptors was reduced in several animal models (45). For example, Fig. 17 shows the results of experiments on rabbits with 2-kidney 1-clip and aortic coarctation hypertension in which there was severe pressure overload resulting in a reduced stroke volume and elevated left ventricular filling pressure. In both models of pressure overload left ventricular decompensation, the concentration of β-adrenergic receptors, measured by ^{3}H-dihydroalprenolol binding, was considerably lower than in the control group. As in Fig. 10, there is a functional correlate; the isoprenaline vs. heart rate dose-response curve is shifted to the right in animals with pressure overloaded left ventricular decompensation, indicating a decrease in the chronotropic sensitivity to isoproterenol.

Several reports have subsequently demonstrated that the density of ventricular myocardial β adrenoreceptors is reduced in patients with congestive heart failure regardless of etiology (46, 47), and that this decrease in β-adrenoceptor number is associated with the reduced sensitivity of myocardial contractile responses to β-adrenergic stimulation (46). Fig. 18 shows that the effect is β_1 selective, since there is decreased responsiveness in the failing hearts to isoproterenol, a nonselective β agonist, and to denopamine, a selective β_1 agonist, with no significant difference between the nonfailing and failing hearts in response to the selective β_2 agonist zinterol (48). The decrease in β_1-receptor-mediated response is not related to a deficit in the adenylate cyclase cAMP system, because cAMP formation and contractile responses to histamine, forskolin, and sodium fluoride are not decreased in congestive heart failure (46, 49).

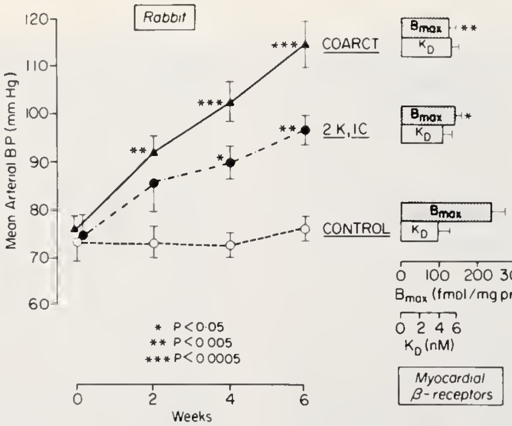


Fig. 17. Mean arterial blood pressure responses in aortic coarctation (COARCT) and two-kidney, one-clip (2K,1C) LV overload in rabbit, with B_{max} (receptor density) and K_D (an index of receptor-ligand affinity) values for ^{3}H-dihydroalprenolol binding to myocardial cell membranes at 6 weeks after surgery. *Reprinted* with permission from Hurwitz et al. (45).

What is the mechanism of the down-regulation of myocardial β_1 adrenoreceptors? The most widely accepted explanation relates to excessive sympathetic tone. Patients with congestive heart failure are known to have high levels of circulating norepinephrine, as well as increased plasma renin activity and arginine vasopressin concentration. The elevated level of plasma norepinephrine is probably a marker for the increased release of norepinephrine into synaptic clefts. When the heart is normal, vasoconstriction induced by the sympathetic nervous system, renin-angiotensin system and by vasopressin may be an appropriate mechanism to support the blood pressure. However, in congestive heart failure, these are counterproductive, leading both to a further detriment in pump performance (due to an increase in afterload and LV wall stress) and to a down-regulation of cardiac myocyte β receptors (50).

Both chronotropic and inotropic responses are mediated by β_1 and β_2 adrenoreceptors in the myocardium, although β_1 adrenoreceptors are present in greater concentrations (51, 52). The β_1 receptor population is more sensitive to downregulation (53). This down-regulatory process can be a rapid, dynamic one. In rats, almost half the myocardial β adrenoreceptors are "lost" within 10 minutes of incubation with isoproterenol (54), and 85% of these "lost" receptors return to the cell surface when the β agonist is removed. The recycling of the β adrenoreceptor to the cell surface is inhibited by monesin and colchicine, implying that the Golgi apparatus and the microtubules are somehow involved in this process (49).

Recently Limas et al. (55) reported a fascinating finding, that sera from patients with idiopathic dilated cardiomyopathy reduced the number of β adrenoreceptors in rat cardiac membranes, and this decrease can be prevented by preincubating the sera with antihuman IgG, indicating the presence of autoantibodies (Fig. 19). We do not know whether this finding will be extended to sera from patients with congestive cardiac failure of other causes, and, if so, whether this is a mechanism which is alternative or additional to the β-adrenoceptor downregulation.

In a study on hearts from normal subjects and patients with idiopathic dilated cardiomyopathy (56), it was found that the number of sarcolemmal α_1-adrenoreceptor binding sites in the diseased hearts was almost twice that in the normal hearts. In the failing hearts the α_1 receptors were shown to be coupled to a GTP-binding protein.

There are probably also changes in muscarinic receptor function. In dogs with experimental heart failure, ligand binding studies using ^{3}H-quiniclidinyl benzilate and LV sarcolemma showed that muscarinic receptor density in heart failure declined 36% from control, with the loss of high affinity agonist binding sites. The functional efficacy of the muscarinic receptor was consequently impaired, as assessed by significantly smaller inhibition of adenylate cyclase by the muscarinic agonist metacholine (57).

Excitation-Contraction Coupling in Heart Failure. There is not much data on the way, qualitatively or quantitatively, that the excitation-contraction coupling process in heart muscle is altered in congestive heart failure. It has been shown that the activity of a 40 000 molecular weight pertussis toxin substrate, probably the α subunit of the inhibitory G protein (α-G_i), is increased by 36% in failing human hearts when compared with nonfailing controls, associated with a 30% decrease in basal as well as 5′-guanylyl imidodiphosphate-stimulated adenylate cyclase activity (58). Neuman et al. (59) described similar findings in the explanted hearts of three patients with end-stage dilated cardiomyopathy (Fig. 20). Recently, using the two techniques of pertussis toxin labelling and immunoblotting techniques, Bohm et al. confirmed that $G_{i\alpha}$ is increased in the hearts of patients with end-stage disease (60), but not, surprisingly, in patients with congestive failure due to coronary artery disease (61).

Further studies in which antibodies have been used to examine failing hearts have indicated that minor decreases in the amount of the active (α) moiety of G_s may be associated with prominent changes in functional activity of this protein in terms of its ability to be activated by GTP (62).

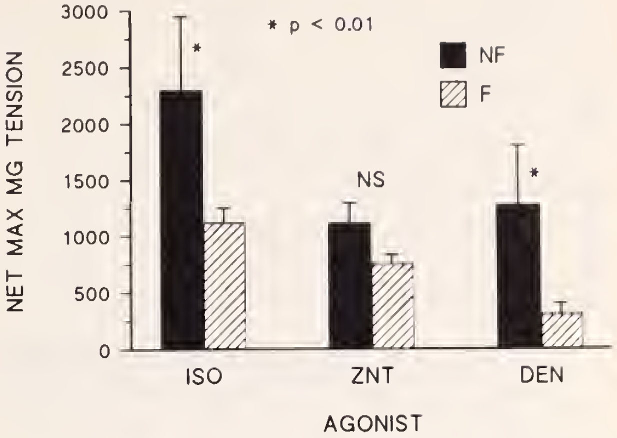


Fig. 18. Tension development in trabeculae from nonfailing (NF) and failing (F) right ventricles in response to stimulation with isoproterenol (ISO; nonselective β agonist), zinterol (ZNT; β_2 agonist) and denopamine (DEN; β_1 agonist). *Reprinted* with permission from Bristow et al. (48).

These studies have given rise to an attempt, by Horn et al. (63), to use peripheral blood mononuclear leucocytes (MNL) as a "tissue" to examine changes in the concentration of G_s in patients with congestive heart failure. Patients with heart failure (New York Heart Association Class III) had 80% less MNL αG_s than controls (Fig. 21), and these levels increased 2.5-fold after treat-

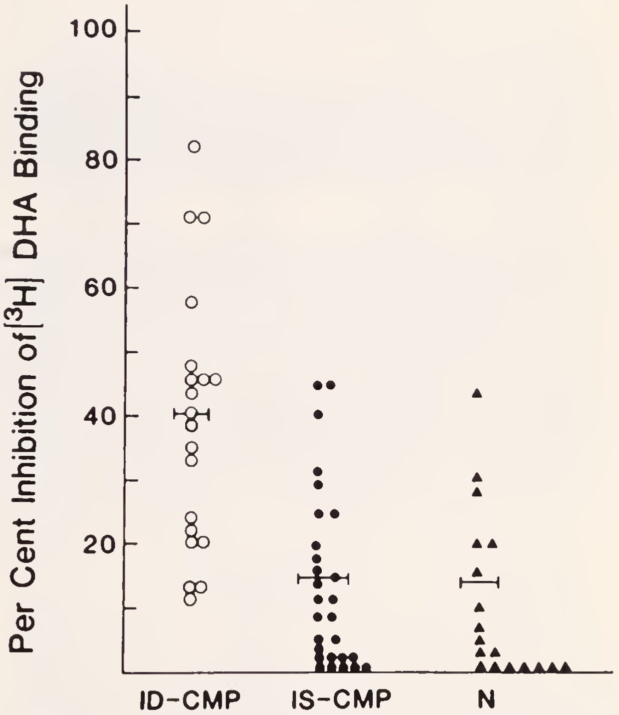


Fig. 19. Effect of sera from patients with idiopathic dilated cardiosympathy (ID-CMP), with ischemic/valvular heart disease (IS-CMP), and with normal cardiac function (N) on the binding of ^{3}H-dihydrolaprenolol ([^{3}H]DHA) to rat cardiac membranes. Percentage of inhibition is calculated from binding to control membranes (preincubated without serum). *Reprinted* with permission from Limas et al. (55).

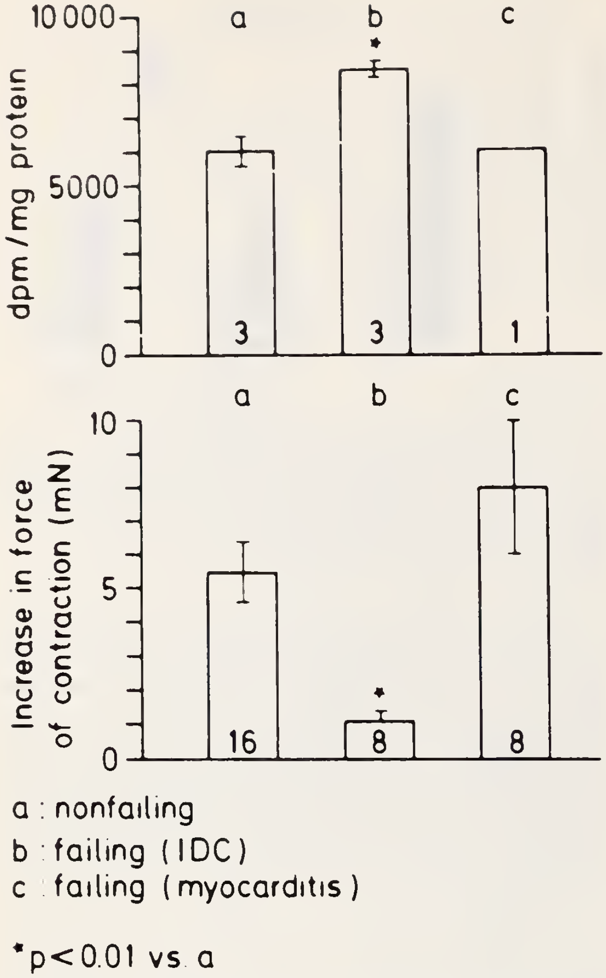


Fig. 20. G_i content (**top**) and contractile response to isoprenaline (**bottom**) of heart tissue from patients with nonfailing (a), failing (idiopathic dilated cardiomyopathy: b), and failing (myocarditis: c) hearts. *Reprinted* with permission from Neuman et al. (59).

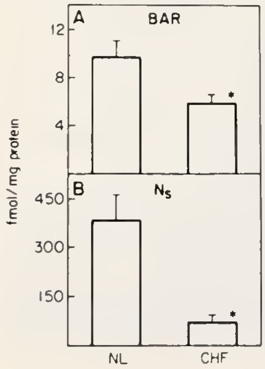


Fig. 21. β Adrenoceptors (BAR, box A) and G_s (N_s, box B) in lymphocytes from normal subjects (NL) and patients with congestive heart failure (CHF). *Reprinted* with permission from Horn et al. (63).

ment with angiotensin converting enzyme inhibitors. Although these are potentially important observations, these data raise a number of problems (64). They include the possibility that these effects may be the result of raised plasma catecholamine levels, and that the method used (cholera toxin labeling) is not an adequate means to quantify G proteins. There are also some worrying conceptual questions, such as: Is congestive heart failure a symptom complex that involves cells throughout the body, as would be implied by a decrease in G_s in MNL? How could treatment with ACE inhibitors raise levels of MNL G_s?

By contrast, Maisel et al. found no difference in lymphocyte cholera toxin substrate (G_s) or pertussis toxin substrate (G_i) between healthy controls and patients with heart failure (65) due to coronary artery disease or cardiomyopathy (66); however, inactivation of αG_i with pertussis toxin enhanced PGE-stimulated cAMP formation in patients with heart failure but not controls. This suggests that there is at least an increase in functional activity of this inhibitory G protein in patients with heart failure.

The consequence of enhanced G_i activity, with or without some defect of G_s, is a deficient production of cAMP. In a study on myocardium in vitro from patients with end-stage cardiac failure (67), peak isometric force generated in response to increased extracellular Ca^{2+} reached control levels, but the time course of contraction and the rate of relaxation were greatly prolonged. The inotropic effectiveness of the β-adrenergic agonist isoproterenol and the phosphodiesterase inhibitors milrinone, caffeine, and isobutylmethylxanthine was markedly reduced. In contrast, the effectiveness of inotropic stimulation with acetylstrophanthidin and the adenylate cyclase activator forskolin was preserved. After a minimally effective dose of forskolin was given to elevate intracellular cAMP levels, the inotropic response of muscle from the failing hearts to phosphodiesterase inhibitors were markedly potentiated. These data suggest that an abnormality in cAMP production may be a fundamental defect in patients with end-stage heart failure, and that this could greatly diminish the effectiveness of agents that depend on cAMP for the production of a positive inotropic effect.

A predicted consequence of decreased cAMP concentration is abnormal intracellular Ca^{2+} handling in these heart cells. Some elegant work from the same authors (68) has provided evidence that this is so. In heart muscle from patients with end-stage cardiac failure, contractions and Ca^{2+} transients of muscles from failing hearts were markedly prolonged, and there was a diminished capacity to restore low resting Ca^{2+} levels during diastole (Fig. 22).

The Cellular-Molecular Basis of Action of Inotropic Drugs in Heart Failure. What does all of the information on alterations in adrenoreceptors and transduction mechanisms in heart failure mean for the pragmatic cardiologist? There is

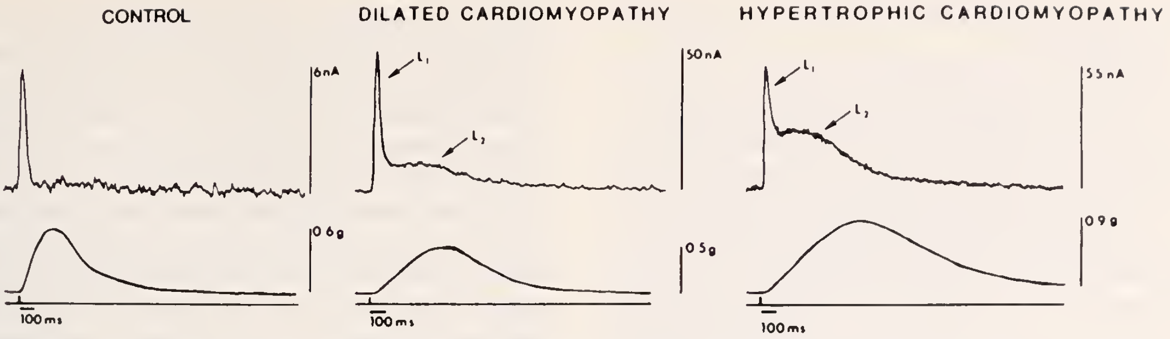


Fig. 22. Intracellular Ca^{2+} transients (upper traces) and contractile tension (lower traces) of heart muscle from controls, and patients with dilated cardiomyopathy and hypertrophic cardiomyopathy. Reproduced with permission from Gwathmey et al. (68).

no question that the elucidation of these membrane-linked and intracellular receptor-contraction coupling events has clarified the mechanisms of action of more traditional drugs for heart failure and has made possible the development of new forms of therapy (69).

There is consistent evidence of an increase in myocardial G_i activity in end-stage heart failure; the studies in lymphocytes are less consistent, and the value of lymphocytes as a surrogate for myocardium is questionable (64, 70, 71), but the idea that ACE inhibitors may be inotropic by virtue of an increase in β receptors and G_s activity (63) is an attractive one.

The increase in G_i activity will impair the efficacy of any agonist acting through adenylate cyclase, and, consequently, it may be predicted that effective inotropic agents in heart failure will be those that will reverse the down-regulation of β adrenoreceptors and increase in G_i activity, such as β blockers (72), and those that act beyond or independent of adenylate cyclase, such as phosphodiesterase inhibitors, "Ca^{2+} sensitizers," Ca^{2+} channel activators, or the old-fash-

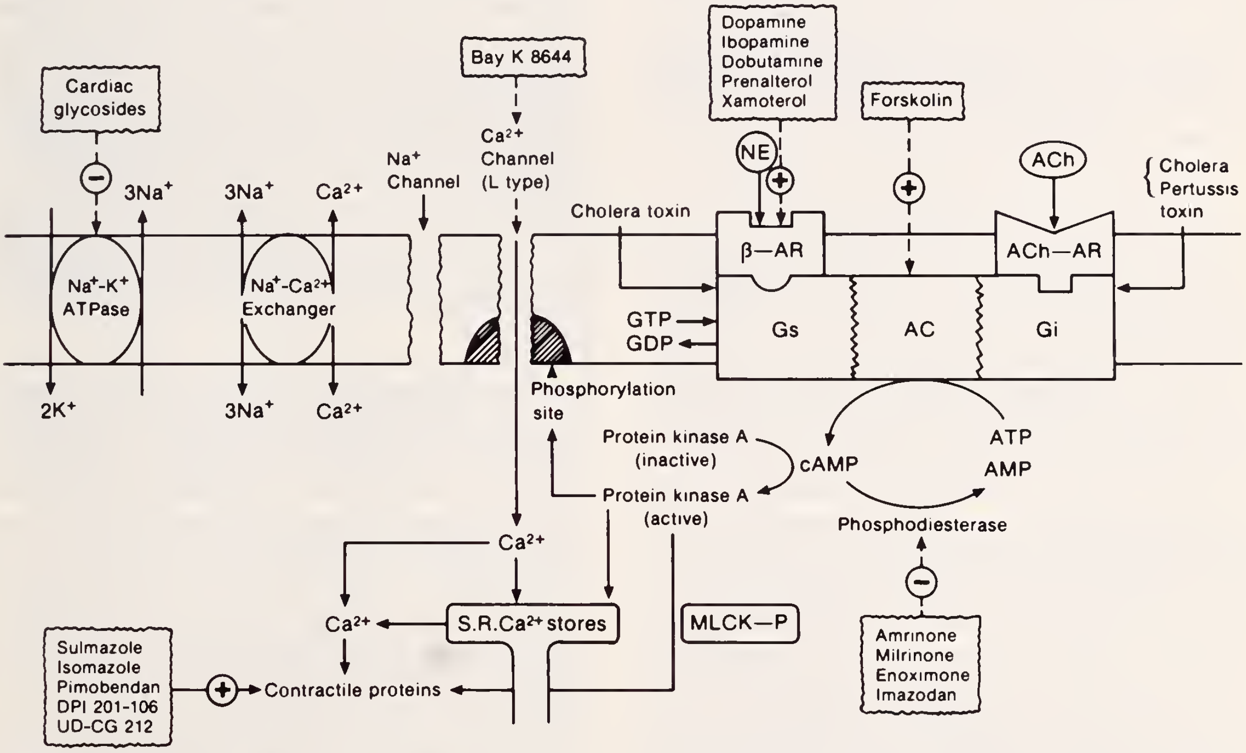

Cardiac glycosides
3Na+
2K+
Na+-K+ ATPase
3Na+
Ca2+
Na+-Ca2+ Exchanger
3Na+
Ca2+
Na+ Channel
Bay K 8644
Ca2+ Channel (L type)
Phosphorylation site
Dopamine
Ibopamine
Dobutamine
Prenalterol
Xamoterol
NE
Forskolin
ACh
Cholera toxin
Cholera
Pertussis
toxin
β—AR
ACh—AR
GTP
GDP
Gs
AC
Gi
Protein kinase A (inactive)
Protein kinase A (active)
cAMP
ATP
AMP
Phosphodiesterase
Amrinone
Milrinone
Enoximone
Imazodan
Ca2+
S.R.Ca2+ stores
MLCK—P
Sulmazole
Isomazole
Pimobendan
DPI 201-106
UD-CG 212
Contractile proteins

Fig. 23. Schematic diagram of main classes of inotropic drugs and their cellular and molecular sites of action, including cardiac glycosides (Na^+-K^+ ATPase inhibitors), dopamine and analogs (β receptor), amrinone, milrinone and analogs (phosphodiesterase inhibitors) and more experimental modalities of treatment, such as Bay K 8644 (Ca^{2+} channel activator), forskolin (adenylate cyclase stimulator), and a number of drugs, such as 3 sulmazole, which are "Ca^{2+} sensitizers."

ioned cardiac glycosides, acting via Na^+-K^+ ATPase (Fig. 23).

Summary. In congestive heart failure the single most consistent finding concerning autonomic receptor function is the downregulation of β_1 receptors which is probably caused by an increase in plasma catecholamine concentration, but also with some evidence that there are anti-β_1-adrenoreceptor autoantibodies. There is also a downregulation of muscarinic receptors, and an increase in the activity of the inhibitory G protein (G_i), with less good evidence for decreased activity of the stimulatory G protein (G_s). There is certainly defective production of cyclic AMP and abnormal calcium handling which may be important in diastolic as well as systolic disfunction.

Still to be determined are the mechanisms by which receptors are up and down regulated, the control of the expression of all of these intermediates, the G proteins, adenylate cyclase, phospholipase C, inositol trisphosphate, diacylglycerol, and perhaps as important, the control of the expression of many of the growth related genes, proto-oncogenes, which are concerned with morphological adaptations to chronic volume or pressure loads in the myocardium. The challenge will then be to use all of this information to design new therapeutic strategies in an area in which the therapeutic yield, particularly with regard to inotropic drugs, has been disappointingly poor. We are at the threshold of a new and exciting era in which many of these unanswered questions will be tackled using the powerful tools of cell and molecular biology.

Questions and Answers

Taubman: Thank you, Dr. Rosendorff, for so elegantly and lucidly making sense out of such a broad and complicated group of topics. I have a question which pertains to the hypertensive models that you discussed. I notice that you presented data from really three or four different models, including cell culture. How does one make sense of which models to use, and how does one really relate this to, say, the human situation?

Clive Rosendorff: All of the animal models that have been developed can be divided into two groups: those which are low renin-angiotensin models, and those which are high renin-angiotensin models. For example, if you give an animal DOCA and salt, you produce essentially a volume-overload model with a low renin-angiotensin expression, and of course all cellular effects will reflect the volume overload status. Conversely, renovascular or renal ischemia animal models are renin-angiotensin dependent, and the pathophysiology and manifestations of the hypertension will be quite different. What about cell cultures? To what extent does a cell which has been passaged many times retain the membrane and cytosolic characteristics of the animal which originally gave rise to it? We know for example that many of the membrane-related protein receptor molecules are lost in cell culture, including α_1 receptors. Clearly, as an exercise in cell biology to understand some of the cellular mechanisms, these models are critical. But whether they translate easily to whole-animal pathology is controversial.

Question: How do you reconcile the fact that hypertension is treated with β-receptor antagonists, when in fact you show that there is downregulation of the β receptors?

Rosendorff: Antagonists probably have several mechanisms of action in hypertension. They reduce the cardiac output, they block the release of renin from the juxtaglomerular cells of the kidney, they reduce norepinephrine release from sympathetic nerve terminals, and they block postjunctional vasodilator β receptors. Clearly these interrelationships are rather complex. What is undeniable is that there is downregulation of β receptors and upregulation of α_1 and α_2 receptors in vascular smooth muscle in hypertension, and the most economical hypothesis is that the upregulation of the receptors which mediate vasoconstriction are somehow involved in the pathogenesis of the disease, and the downregulation of β receptors is a consequence of the disease.

Question: My second question has to do with the IP_3 response to α-adrenergic stimulation of the heart. To my knowledge the existence of α receptors in the heart has not been definitively proven at a molecular level. Can you say something more about that?

Rosendorff: Let me say a little about a model which we have used. IP_3 is released in response to balloon stretch of the left atrium, presumably as a result of α_1 activation. This model is not really congestive heart failure, but I think it mimics the atrial myocardial stretch that occurs in left ventricular failure. Under those circumstances, one can show an increased rate of phosphorylation of inositols in the atrium, indirect evidence of activation of α_1 receptors. Whether that is what happens in congestive heart failure, I don't know.

Question: Are you aware of any data, or if not, can you speculate on what you would expect the functional levels of β-adrenergic receptor kinase to be in the downregulated myocardium?

Rosendorff: Yes. β-adrenergic receptor kinase is a kinase which phosphorylates the β receptor and thereby inactivates it. I am not aware of any data on that subject in hypertension. The subject of desensitization of β receptors is a fascinating one and has been particularly well studied for the stimulation of cAMP levels by membrane β-adrenergic receptors. Apparently desensitization involves phosphorylation of the β receptor by at least two kinases, protein kinase A and the β-adrenoreceptor kinase, which are activated under different desensitization conditions. This phosphorylation process then uncouples the receptors from the stimulatory G protein, G_s (73).

References

1. Hales S. Statical essays: containing haemostaticks. London 1733.
2. Goldstein DS. Plasma catecholamines and essential hypertension. Hypertension 1983; 5:86–99.
3. Reis DJ, Granata AR, Joh TH, Ross CA, Rugiero DA, Park DH. Brain stem catecholamine mechanisms in tonic and reflex control of blood pressure. Hypertension 1984; II 7–15.
4. Rosendorff C. Alpha-adrenoreceptors in hypertension. J Cardiovasc Pharmacol 1986; (S2):S3–S7.
5. Cambridge D, Davey MJ, Massingham R. Prazosin, a selective antagonist of postsynaptic alpha-adrenoceptors. Br J Pharmacol 1977; 59:514P–515P.
6. Davey MJ. Alpha adrenoceptors—an overview. J Mol Cell Cardiol 1986; 18 (suppl 5):1–15.
7. Rosendorff C, Susanni E, Hurwitz ML, Ross FP. Adrenergic receptors in hypertension: radioligand binding studies. J Hypertension 1985; 3:571–581.
8. Michel MC, Brodde O-E, Insel PA. Peripheral adrenergic receptors in hypertension. Hypertension 1990; 16:107–120.
9. Raymond JR, Hnatowich M, Lefkowitz RJ, Caron MG. Adrenergic receptors: models of regulation of signal transduction processes. Hypertension 1990; 15:119–131.
10. Farmer SG. Noradrenaline and ATP-contransmitters. Trends Pharmacol Sci 1985; 6(1):10–11.
11. Rosendorff C, U'Prichard DC, Hurwitz ML. Alpha-noradrenergic receptor binding sites in bovine aorta. Basic Res Cardiol 1981; 76:536–539.
12. Susanni EET, Ross FP, Scriven DRL, Rosendorff C. Baboon erythrocyte ghosts contain beta-adrenergic receptors. Am J Physiol 1985; 249(1, Pt 1):C15–C19.
13. Grollman A. A simplified procedure for inducing chronic renal hypertension in the mammal. Proc Soc Exp Biol Med 1944; 51:102–104.
14. Hurwitz ML, Rosendorff C. Cardiovascular adrenoceptor number and function in experimental hypertension in the baboon. J Cardiovasc Pharmacol 1985; 7(56):S172–S177.
15. Weidman P, Grimm M, Meier A, Glück Z, Keusch E, Minder I, Beretta-Piccoli C. Pathogenic and therapeutic significance of cardiovascular pressor reactivity as related to plasma catecholamines in borderline and established essential hypertension. Clin Exp Hypertens 1980; 2:427–449.
16. Bertel O, Bühler RF, Kiowski W, Lütold BE. Decreased beta-adrenoceptor responsiveness as related to age, blood pressure, and plasma catecholamines in patients with essential hypertension. Hypertension 1980; 2:130–138.
17. Michel MC, Daul A, Galal O, Wang X-L, Brodde O-E. Are alpha- and beta-adrenoceptor changes in patients with essential hypertension genetically determined? J Hypertension 1988; 6(S4):S575–S577.
18. Eid H, de Champlain J. Increased inositol monophosphate production in cardiovascular tissue of DOCA-sal hypertensive rats. Hypertension 1988; 12:122–128.
19. Takata Y, Yoshida Y, Ozaki S, Kato H. Increases in vascular α_1-adrenoceptor affinity and PI turnover in DOCA-salt hypertensive rats. Eur J Pharmacol 1989; 164:365–368.
20. Benze J, Yang H-Y, Griffith VJ, Rosendorff C. Angiotensin-II induced phosphorylation of myosin light chain in vascular smooth muscle cells from spontaneously hypertensive and normotensive rats. Cardiovasc Res 1992 (in press).
21. Rosendorff C, Kalliatakis B, Radford HM, Patton J. Age dependence of the pressor sensitivity to noradrenaline and angiotensin II during calcium channel blockade in hypertensive patients. J Cardiovasc Pharmacol 1988; 12:S69–S71.
22. Tsien RW, Tsien RY. Calcium channels, stores, and oscillations. Ann Rev Cell Biol 1990; 6:715–760.
23. Takuwa N, Takuwa Y, Yanagisawa M, Yamashita K, Masaki T. A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. J Biol Chem 1989; 264(14):7856–7861.
24. Brown KD, Littlewood CJ. Endothelin stimulates DNA synthesis in Swiss T3T cells: synergy with polypeptide growth factors. Biochem J 1989; 263(3):977–980.
25. Komuro I, Kurihara H, Sugiyama T, Takaku F, Yazaki Y. Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. FEBS Lett 1988; 238:249–252.
26. Hirata Y, Takagi Y, Fukuda Y, Marumo F. Endothelin is a potent mitogen for rat vascular smooth muscle cells. Atherosclerosis 1989; 78(2–3):225–228.
27. Bloom DS, McCalden TA, Rosendorff C. The effect of hypercholesterolemic plasma on vascular sensitivity to noradrenaline. Brit J Pharmacol 1975; 54:421–427.
28. Rosendorff C, Hoffman JIE, Verrier E, Rouleau JR, Boerboom LE. Cholesterol sensitizes coronary vessels to norepinephrine. Circ Res 1981; 48:320–329.
29. Hurwitz M, Rosendorff C, Jarvis AC, Revington JP. Effect of sodium loading and depletion in high cholesterol diets on alpha noradrenergic receptors in a variety of rabbit tissue. S Afr J Sci 1982; 78:82–83.
30. Katzeff I, Levin G, Hurwitz ML, Rosendorff C. Dietary fatty acid saturation affects coronary vascular resistance and beta-adrenoreceptors in the rat heart. Prog Lipid Res 1986; 25:479–483.
31. Sachinidis A, Locher R, Steiner A, Mengden T, Vetter W. Effect of low-density lipoprotein on intracellular calcium, intracellular pH and DNA synthesis in cultured vascular smooth muscle cells. J Hypertension 1989; 7 (Suppl 6):S116–S117.
32. Ransone LJ, Verma IM. Nuclear proto-oncogenes fos and jun. Ann Rev Cell Biol 1990; 6:539–557.
33. Verma IM. Proto-oncogene fos: a multifaceted gene. Trends Genet 1986; 2(4):93–96.
34. Taubman MB, Berk BC, Izumo S, Tsuda T, Alexander RW, Nadal-Ginard B. Angiotensin II induces c-fos mRNA in aortic smooth muscle. J Biol Chem 1989; 264: 526–530.

35. Naftilan AJ, Pratt RE, Eldridge CS, Lin HL, Dzau VJ. Angiotensin II induces c-fos expression in smooth muscle via transcriptional control. Hypertension 1989; 13: 706–711.
36. Levy BI, Michel J-B, Salzmann J-L, Azizi M, Poitevin P, Safar M, Camilleri J-P. Effects of chronic inhibition of converting enzyme on mechanical and structural properties of arteries in rat renovascular hypertension. Circ Res 1988; 63:227–239.
37. Asmar RG, Journo HJ, Lacolley PJ, Santoni JP, Billaud E, Levy BI, Safar ME. Treatment for one year with perindopril: effect of cardiac mass and arterial compliance in essential hypertension. J Hypert 1988; 6:S33–S39.
38. Christensen KL, Jespersen LT, Mulvany MJ. Development of blood pressure in spontaneously hypertensive rats after withdrawal of long-term treatment related to vascular structure. J Hypertension 1989; 7:83–90.
39. Chidsey CA, Braunwald E, Morrow AG. Catecholamine excretion and cardiac stress of norepinephrine in congestive heart failure. Am J Med 1965; 39:442–451.
40. Thomas JA, Marks BH. Plasma norepinephrine in congestive heart failure. Am J Cardiol 1978; 41:233–243.
41. Levine TB, Francis GS, Goldsmith SR, Simon A, Cohn JN. Activity of the sympathetic nervous system and renin angiotensin system assessed by plasma hormone levels and their relationship to hemodynamic abnormalities in congestive heart failure. Am J Cardiol 1982; 49: 1659–1666.
42. Daly PA, Sole MJ. Myocardial catecholamines and the pathophysiology of heart failure. Circulation 1990; 82 (Suppl I):I-35–I-43.
43. Vatner DE, Lee DL, Schwarz KR, Longabaugh JP, Fujii AM, Vatner SF, Homcy CJ. Impaired cardiac muscarinic receptor function in dogs with heart failure. J Clin Invest 1988; 81:1836–1842.
44. Prokopczuk A, Pytkowski B, Lawartowski B. Effect of acetylcholine on calcium efflux from atrial myocardium. Eur J Pharmacol 1981; 70:1–6.
45. Hurwitz ML, Rosendorff C, Mills M, Houghton D, Bethlehem A. Myocardial beta-receptors in experimental renal and aortic coarctation hypertensive rabbits. J Hypertension 1984; 2(3):85–87.
46. Bristow MR, Ginsburg R, Minobe W, et al. Decreased catecholamine sensitivity and beta-adrenergic receptor density in failing human hearts. N Eng J Med 1982; 307:205–211.
47. Ruffolo RR, Kopia GA. Importance of receptor regulation in the pathophysiology and therapy of congestive heart failure. Am J Med 1986; 80 (Suppl 2B):67–72.
48. Bristow MR, Hershberger RE, Port JD, et al. β-Adrenergic pathways in nonfailing and failing human ventricular myocardium. Circulation 1990; 82 (Suppl I):I-12–I-25.
49. Baumann G, Mercader D, Busch U, et al. Effects of the H_2-receptor agonist impromidine in human myocardium from patients with heart failure due to mitral and aortic valve disease. J Cardiovasc Pharmacol 1983; 5: 618–625.
50. Stiles GL, Taylor S, Lefkowitz RJ. Human cardiac beta-adrenergic receptors: subtype heterogenicity delineated by direct ligand binding. Life Sci 1983; 33:467–473.
51. Packer M. Role of the sympathetic nervous system in chronic heart failure; a historical and philosophical perspective. Circulation 1990; 82 (Suppl 2):I-1–I-6.
52. Williams RS. Selectivity of prenalterol for adrenergic receptor subtypes: a potential mechanism of inotropic selectivity. J Cardiovasc Pharmacol 1983; 5:266–271.
53. Bristow MR, Ginsburg R, Umans V, et al. $Beta_1$- and $beta_2$-adrenergic receptor subpopulations in nonfailing and failing human ventricular myocardium: Coupling of both receptor subtypes to muscle contraction and selective $beta_1$-receptor down regulation in heart failure. Circ Res 1986; 59:297–309.
54. Limas CJ, Limas C. Rapid recovery of cardiac beta-adrenergic receptors after isoproterenol-induced "down"-regulation. Circ Res 1984; 55:524–531.
55. Limas CJ, Goldenberg IF, Limas C. Autoantibodies against beta-adrenoceptors in human idiopathic dilated cardiomyopathy. Circ Res 1989; 64:97–103.
56. Vago T, Bevilaqua M, Norbiato G, et al. Identification of $alpha_1$-adrenergic receptors on sarcolemma from normal subjects and patients with idiopathic dilated cardiomyopathy characteristics and linkage to GTP-binding protein. Circ Res 1989; 64:474–481.
57. Vatner DE, Lee DL, Schwarz KR, Longabaugh JP, Fujii AM, Vatner SF, Homcy CJ. Impaired cardiac muscarinic receptor function in dogs with heart failure. J Clin Invest 1988; 81:1836–1842.
58. Feldman AM, Cates AE, Veazey WB, et al. Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in the failing human heart. J Clin Invest 1988; 82:189–197.
59. Neuman J, Schmitz W, Scholz H, Von Meyerinck L, Doring V, Kalmar P. Increase in myocardial G_i-proteins in heart failure. Lancet 1988; 2:936–937.
60. Bohm M, Gierschik P, Schnabel P, Erdmann E. Myocardial beta-adrenoceptors and inhibitory G-protein in myocardial biopsies and in explanted hearts from patients with dilated cardiomyopathy. Cardioscience 1990; 1(2):109–117.
61. Bohm M, Gierschik P, Jakobs KH, et al. Increased $G_{i\alpha}$ in human hearts with dilated but not ischemic cardiomyopathy. Circulation 1990; 82:1249–1265.
62. Ransnas LA, Hjalmarson A, Insel PA. Dilated cardiomyopathy is associated with an impaired activation of the stimulatory G protein, G_s, by GTP in heart membranes (abstract). Circulation 1988; 78 (suppl II):II-178.
63. Horn EM, Corwin SJ, Steinberg SF, et al. Reduced lymphocyte stimulatory guanine nucleotide regulatory protein and beta-adrenergic receptors in congestive heart failure and reversal with angiotensin converting enzyme inhibitor therapy. Circulation 1988; 78:1373–1379.
64. Insel PA, Ransnas LA. G proteins and cardiovascular disease. Circulation 1988; 78:1511–1513.
65. Maisel AS, Michel MC, Insel PA, Ennis C, Ziegler MG, Phillips C. Pertussis toxin treatment of whole blood: a novel approach to assess G protein function in congestive heart failure. Circulation 1990; 81:1198–1204.
66. Michel MC, Maisel AS, Brodde OE. Mitigation of β_1 and or β_2-adrenoceptor function in human heart failure. Br J Clin Pharmacol 1990; 3 (Suppl 1):37S–42S.
67. Feldman MD, Copelas L, Gwathmey JK, et al. Deficient production of cyclic AMP: pharmacologic evidence of an important cause of contractile dysfunction in patients with end-stage heart failure. Circulation 1987; 75:331–339.
68. Gwathmey JK, Copelas L, MacKinnon R, Schoen FJ, Feldman MD, Grossman W, Morgan JP. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. Circ Res 1987; 61: 70–76.

69. Wetzel B, Hauel N. New cardiotonic agents—a promising approach for the treatment of heart failure. Trends Pharmacol Sci 1988; 9:116–170.
70. Brodde OE, Michel MC, Gordon E, Sandoval A, Gilbert EM, Bristow MR. β adrenoceptor regulation in the human heart: can it be monitored in circulating lymphocytes. Eur Heart J 1989; 10 (Suppl B):2–10.
71. Editorial. G proteins and heart failure. Lancet 1990; 336: 1412–1414.
72. Heilbrunn SM, Shah P, Bristow MR, Valantine HA, Ginsburg R, Fowler MB. Increased β-receptor density and improved hemodynamic response to catecholamine stimulation during long term metoprolol therapy in heart failure from dilated cardiomyopathy. Circulation 1989; 79:483–490.
73. Hausdorff WP, Caron MG, Lefkowitz RJ. Turning off the signal: desensitization of β-adrenergic receptor function. FASEB J 1990; 4:2881–2889.

Grand Rounds in Cardiology

Newer Aspects of Neuroendocrine Control in Heart Failure

ROBERT J. CODY, M.D.

SOME of the discussion of neuroendocrine factors in congestive heart failure I present here is reflective and some of it is hard data. This presentation will not come up with answers, so much as formulate the questions that need answers. I think that some of the newer neurohormonal studies in heart failure have brought some new twists on old themes. In the early 1980s much was said about how heart failure produces increases in neurohormonal factors, and in the not-so-subtle data from recent clinical trials, what is apparent is that—at least in mild heart failure—these factors may not be increased.

My aim here is to give some perspective to that data by reviewing the issues raised by studies of neurohormonal factors, suggesting we think of these data as a discovery stage in the assessment of modulating factors, the identification of surrogates of neurohormonal activity, and the assessment of how we judge the clinical impact of these factors, and then, based on this perspective, to try to decide if newer insights or a greater understanding of these factors can be gained.

The Discovery Stage: Initial Observations

The two neurohormonal factors that have been longest associated with congestive heart failure are the renin-angiotensin system, of which our knowledge goes back about 45 years, and abnormal sympathetic nervous system activity—autonomic nervous system activity—of which our knowledge goes back almost as long. Our knowledge of these two factors constitutes the grandfathering of all we now know.

Prior to being at Ohio State, I worked at Cornell University for about eight years with Spencer Kubo, John H. Laragh, Steven A. Atlas, and Jean Sealey, investigating many factors related to neurohormonal activity. One of our first assessments in the early 1980s was of plasma renin activity in patients with congestive heart failure compared to normal subjects (Fig. 1). The normal subjects were stimulated, either by upright posture or upright posture plus low-sodium diet. Compared to normal stimulated subjects, on average, renin system activity could be considered normal—if one looks only at the mean values. What was striking to me was the range of renin system activity in congestive heart failure. The levels in the range of 100 ng/mL/hr are consistent with the most severe renal artery stenosis. The lowest levels are barely measurable, 0.1 ng/mL/hr, and such levels typically occur only in primary hypersecretion of aldosterone. In patients not receiving diuretics, and whose sodium intake is normal, the renin level is the lowest. These ten-year-old data suggest that renin levels are not the same in all patients with heart failure.

At the same time I was interested in autonomic activity. A few seminal papers, the earliest one by Thomas and Marks dating from the late 1970s, had examined norepinephrine levels in congestive heart failure (1). Subsequent to that paper, we were able to show a good relationship between plasma norepinephrine and cardiac in-

---

Adapted from the author's Grand Rounds in Medicine presentation at the Mount Sinai Medical Center on October 15, 1991, revised and updated April 1992. From the Department of Medicine, Division of Cardiology, Ohio State University Medical College, Columbus. Address reprint requests to the author, James Hay and Ruth Jansson Wilson Professor of Medicine, Research Director, Division of Cardiology, Ohio State University Medical College, 6th floor Means Hall, 1654 Upham Drive, Columbus, Ohio 43210.

dex (2). We were able to show, for instance, that under baseline conditions, plasma norepinephrine levels tended to be high, inversely related to cardiac index, and after two months of captopril therapy, norepinephrine levels were favorably reduced as the cardiac index increased.

That relationship between norepinephrine levels and cardiac index is an important observation, but it needs to be put into the context of a larger clinical story. In work published by Jay Cohn and his colleagues (3), the researchers looked at plasma norepinephrine in a larger number of patients in a predictive way and attempted to evaluate the results in terms of prognosis. It was clear that subgroup analysis of the values could yield insight into the probability of survival. Patients with the highest norepinephrine levels had the worst survival, and those whose values were just slightly elevated compared to normal did at least reasonably well in the long haul.

Modulating Factors

The theme I want to develop here is that all neurohormones are not the same; they all respond to different stimuli, differences in milieu. Some of the factors that can influence neurohormonal activity are

- severity of heart failure
- age
- medical therapy
- sodium status (pertinent to renin, for example)

Coexistent medical therapy is probably one of the biggest factors affecting the background of neurohormonal activity. Most of the neurohormonal activity was measured against the background of digoxin and diuretics, and even in some cases vasodilator therapy. I don't want to come down too hard on that. Obviously, these parameters were assessed in sick patients, but I think perhaps that the confusion or cynicism that might start to evolve from some of the more recent observations about neurohormonal factors will be the result of not appreciating some of these modulating factors that readily explain discrepancies.

One example of a modulating factor is the influence of sodium status on renin system activity. To specialists in hypertension, this knowledge is decades old; it is old hat, but it did not filter into the study of heart failure for some time. I review here a couple of examples of studies that started to demonstrate the influence of sodium status relating to one system, the renin system.

In a paper published from Cornell in 1986, our group examined responses of the renin system to sodium intake in patients with heart failure (4). Our method was not unlike that utilized in previous studies of hypertension. All subjects were patients with functional class 3 or 4 chronic heart failure. Following withdrawal of medication, patients were randomly assigned to one week of either a 100 mEq or a 10 mEq sodium diet, switching to the alternate sodium diet the following week (Fig. 2). On the 10 mEq sodium diet, mean values of plasma renin activity were 7–8 ng/mL/hr, levels I think most physicians would feel are increased, especially now because of what we are seeing in mild heart failure; certainly the upper value of 10 would be considered

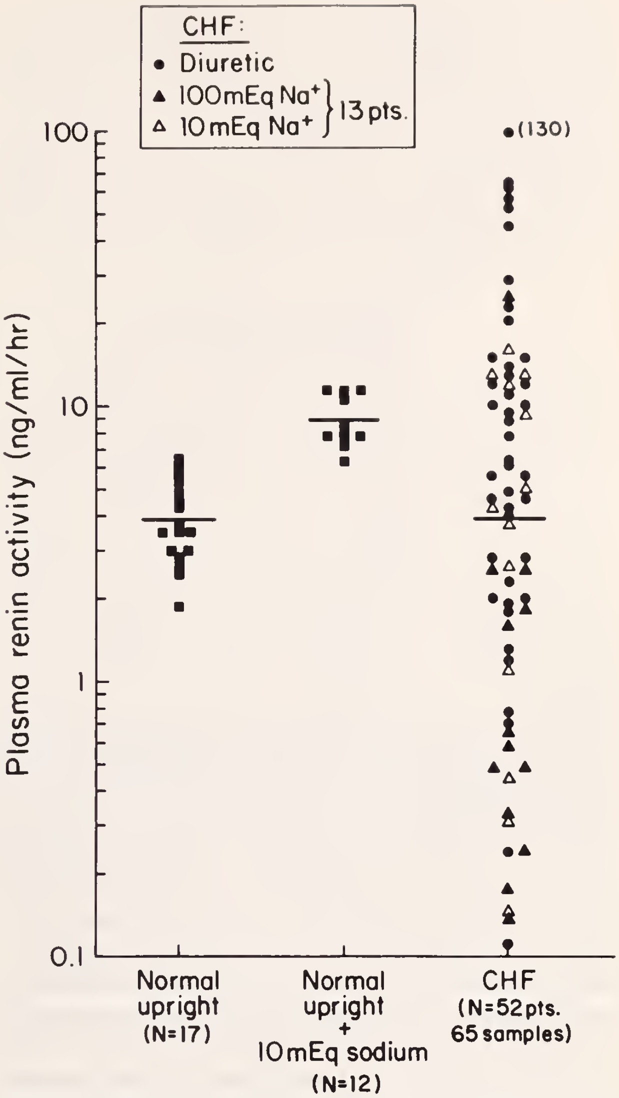
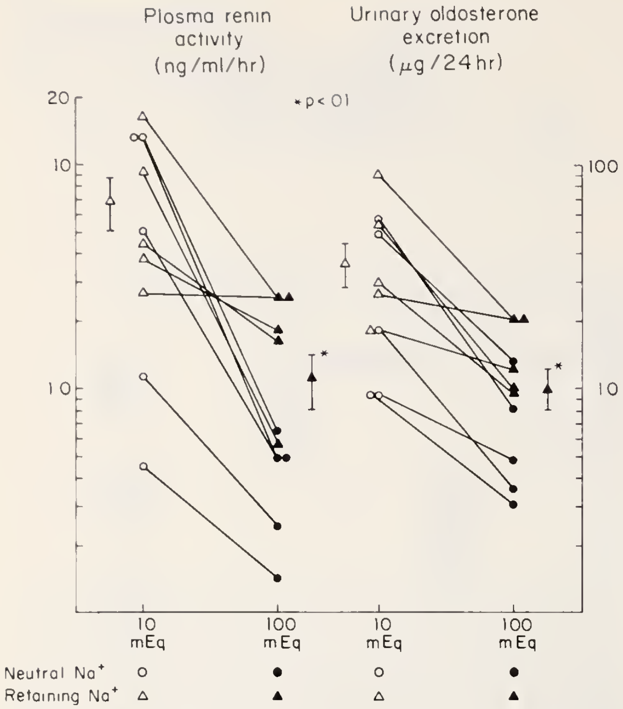


Fig. 1. Range of plasma renin activity in chronic heart failure (CHF) patients under varied sodium conditions, contrasted with normal subjects. *Adapted* from Cody RJ, Laragh JH. The renin-angiotensin-aldosterone system in chronic congestive heart failure: pathophysiology and implications for treatment. In: Drug Treatment of Heart Failure 1988; 79–104.



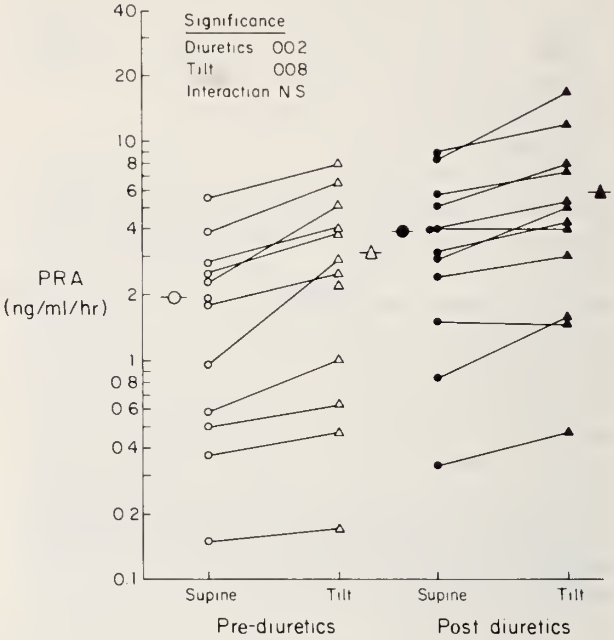


Fig. 2. **Left** Influence of sodium intake on plasma renin activity and urinary aldosterone in group of patients with moderate to severe heart failure. Circles, subjects with neutral sodium balance; triangles, sodium retainers. Changing from 10 to 100 mEq sodium diet produced suppression of renin system, the greatest suppression occurring in those patients achieving neutral sodium balance. *Reprinted* from ref. 4.

Fig. 3. **Right** In mild untreated heart failure, plasma renin activity was normal, and increased during head-up tilt. Following one month of diuretic treatment, baseline renin activity increased. *Reprinted* from ref. 6.

high. But even during the 10 mEq sodium diet, consistent with diuretic therapy, plasma renin demonstrated a wide range, values from about 15 to 0.15 ng/mL/hr, the latter being an abnormally low level.

Put in the context of a patient with severe heart failure, the observation has to be considered striking. Comparing that data with the 100 mEq sodium diet, which is only 2–3 grams, makes clear that all these patients were able to suppress their renin activity. I was always impressed by that. These data demonstrate that the macula densa signal for renin release is intact even in moderate to severe heart failure. This means that the renin secretory mechanism potentially can be maintained, if one can get sodium to the distal nephron. Of course, that is the big problem, one we still have not resolved.

During the 100 mEq sodium diet, patients were divided into groups of those who achieved neutral sodium balance (they took in 100 mEq and basically excreted 100 mEq), and those who continued to avidly retain sodium, excreting less than 20 mEq sodium. The subjects who achieved a neutral balance had the lowest renin activity, which is further proof that if the sodium is delivered to the distal nephron, the renin signal from the macula densa is turned off. The sodium-retaining patients still have relatively higher renin activity than the neutral balance group, and even though the patient numbers are small, subgroup analysis did show that to be significantly different.

Bayliss and co-workers in 1987 (5) studied renin activity and rest and exercise before and after treatment with diuretics. They studied patients with fairly severe heart failure not being treated with diuretics who exercised. The patients were restudied after diuretic therapy of about a month. In the presence of diuretics, plasma renin activity substantially increased, again confirming that either sodium restriction or diuretics is associated with an increased level of plasma renin. So under resting conditions, renin went up, and with maximal exercise renin also went up. Under resting conditions without diuretics, the patients demonstrated relatively little plasma renin activity (at least two patients had levels less than 0.5 ng/mL/hr), despite severe heart failure.

In a paper we published in 1987, looking at mild heart failure, we changed the environment a little bit (6). In that study, patients primarily had functional class II failure. We found that the renin system was not activated in early stages of heart failure (Fig. 3). Baroreceptor stimulation of the renin system during head-up tilt was relatively preserved. After diuretic therapy, basal plasma renin was augmented, and the degree of increase in the renin system during tilt was not further augmented—additional evidence that diuretics stimulate the renin system under baseline conditions.

To put another twist on this, we now start to get into issues related to severity of failure and diuretics. The first paper that came out of the Studies of Left Ventricular Dysfunction (SOLVD) was published by Gary Francis and co-workers (7). The hormonal work was primarily by Claude Benedict in Texas. I think one of the greatest things about this study was its demonstration that investigators from a large number of centers could collect plasma samples for neurohormonal factors, have them analyzed in a central laboratory, and identify consistency of quality and data acquisition across all the centers. So I think one of the most important things about this study was that it demonstrated that assessing such parameters in a large multicenter study was feasible. What they demonstrated was perhaps unusual only because of the population in which it was demonstrated.

For plasma norepinephrine, renin, atrial natriuretic factor (ANF), and vasopressin, they compared control subjects with one group in a prevention arm and another in a treatment arm of the study and demonstrated a stepwise increase in norepinephrine. The prevention arm consisted of patients with essentially asymptomatic left ventricular dysfunction who had not yet developed overt clinical findings of failure; the patients in the treatment arm already had symptomatic heart failure. Both groups were followed prospectively. In essence the treatment group had symptomatic failure and higher norepinephrine values (and higher ANF values) than the group with asymptomatic healthy dysfunction.

ANF, which I discuss below, is one of the good guys, a vasodilator and a diuretic substance whose plasma level increases as atrial wall tension increases. If nothing else, it is a harbinger of atrial distention.

Plasma renin values also went up, particularly between the prevention and the treatment groups. And vasopressin values also rose, but about these values I add a caution: the changes in values of plasma renin and of vasopressin, although statistically significant, are actually pretty tight, and compared to the "normal," at least in terms of what we measure in normal populations, are not really all that different. The statistical significance may hold because of the large number of patients in this study. But I think one would be hard put to identify the biological significance of a 0.1 increment in plasma renin values—of 0.5 versus 0.6 ng/mL/hr. In fact, patients taking diuretics were factored out of the treatment group; this difference was no longer significant. So in essence the plasma renin activity and the vasopressin levels are "normal."

Surrogates of Neurohormonal Factors

About 8 to 10 years ago, I was skeptical of the significance we could attach to a single determination of plasma hormonal levels, and I began to look at the possibility of surrogates for neurohormonal factors. A measure of neurohormonal activity would be useful; when I first started to talk about this, people would say, "How do I measure renin? What does it mean?" It became clear that not everybody can do it, and maybe not everybody should do it. The issue becomes, can we identify surrogates of neurohormonal activity, some value that will give us a quick bedside assessment of some neurohormonal factors? If we cannot make a specific statement about an individual value, we might be able to make some value judgment about these systems. I want to touch on two such surrogates, one well-established and one new.

Hyponatremia. First, hyponatremia is known to be possible evidence of increased vasopressin activity. More important, in the heart failure population there is certainly plenty of evidence that hyponatremia is a reflection of increased renin system activity.

The relationship of hyponatremia and heart failure was first shown in 1970 by Brown and Robertson (8) in Glasgow, Scotland. They used a biological assay for plasma renin levels, and demonstrated an inverse relationship between serum sodium and plasma renin. They were able to distinguish those patients who had been treated from those who were untreated, and showed that patients treated with digoxin were in the normal-sodium, low-renin range. Several papers in the early 1980s looked at this issue, but I think one of the important contributions of Milton Packer was that he took it a step further (9). Utilizing a large number of patients, predictive statements regarding mortality could be made, but for any particular patient, such predictions are difficult. Even within larger patient groups, a patient with a plasma renin of 2 ng/mL/hr can have a serum sodium ranging from abnormal to almost normal. But the important conclusion is the overall picture in respect to predicting survival during the subsequent year. If the patient had a serum sodium level of 128 mEq/L or was left untreated, that patient was not going to do as well as a patient who has a serum sodium level of 142 mEq/L.

An additional observation which enhanced the predictability of the relationship between serum sodium and renin values in patients with heart failure was the patient's response to therapy with an ACE inhibitor, which blocked the renin system. Thus if hyponatremia is a marker for the renin system, blocking the renin system should also have a favorable effect on the marker. So hyponatremia became, I think, one of the best-documented surrogates of a neurohormonal abnormality and a guide we can still use at the bedside.

Spectral Analysis of Heart Rate Variability. The second surrogate of neurohormonal activity is spectral analysis of heart rate variability, a new assessment of neural activity (10). In any regular biological signal, one can look at the frequency of that signal. Since the heart rate is repetitive and cyclic, one can identify patterns of repetitiveness which would reflect both sympathetic and parasympathetic activity.

Such work is currently being conducted at Ohio State, and the lion's share of the credit for this information goes to my close colleague Philip F. Binkley (11). We have been working on it for about three years now. The key thing is that spectral analysis of heart rate variability reflects not only sympathetic activity but also parasympathetic activity.

When we first started to look at catecholamines in the early 1980s (12), it became clear that plasma norepinephrine was a great handle on sympathetic activity, but we really could not get a handle on the parasympathetic activity. It was already clear at that time that parasympathetic activity might be as important, even more important, than sympathetic activity in the pathogenesis of heart failure. One can transform the R-R variability into a frequency domain instead of a time domain to analyze it for power content. This power content is a reflection of simultaneous sympathetic and parasympathetic activity. The patient with heart failure has the low-frequency sinusoidal response. What is missing, however, is all the high-frequency activity in between. It may be hard to tell from the background especially, but the heart rate in persons with heart failure is clearly faster than that of the normal subject, and the high-frequency variability is missing. This is consistent with marked reduction or absence of parasympathetic activity. A patient with congestive heart failure has virtually no high-frequency greater than 0.1 Hz, so that the parasympathetic activity is gone. Sympathetic activity, less than 0.1 Hz, is represented by a high spike that is as much as a full order of magnitude greater than in the normal subject.

These findings in heart failure are consistent with increased sympathetic tone and decreased parasympathetic tone. To prove this, data were gathered in normal subjects given atropine; the high frequency bands above 0.1 Hz disappeared, and the power density spectrum was what one would see in heart failure. This surrogate approach to autonomic activity is currently being pursued by our group and others.

Clinical Impact

Up to this point, I have discussed the kind of physiology that has fueled many a career over the last several decades. But the important thing is relevance to congestive heart failure. The clinical relevance of some of these observations can be identified in several ways.

The first is to utilize a pharmacologic probe or marker for a given neurohormonal pathway, to try to see what happens when the pathway is either augmented or blocked. Obviously, the best example of that approach in heart failure is the use of ACE inhibitors. In contrast, one area still not adequately explored is beta adrenergic blockers. By the early to mid 1990s, as results of some of the larger multicenter studies are revealed, we will know more about blockers of the autonomic system in relation to outcome.

The second approach is to assess the outcome of clinical trials that utilize these probes. So if everything I have said up to now is reasonable and can be accepted as meaningful, then anything we say or can do with these pharmacologic probes can be put into a relevant context.

In terms of clinical impact, I think it is fairly well established that interruption of the renin system has a favorable clinical outcome. In contrast, whether our understanding of atrial natriuretic factor can produce a similar impact is not yet established. However, it took some 35 years after identification of increased renin secretion to establish its relevance in heart failure. In the subsequent decade, its clinical relevance was best clarified with studies using ACE inhibitors.

I think the issue we are now trying to identify is the most important mechanism of action of these neurohormonal inhibitors. Is it the endocrine action? Is it the paracrine-autocrine action? In other words, what are the local effects of these systems? Are there effects on protein production

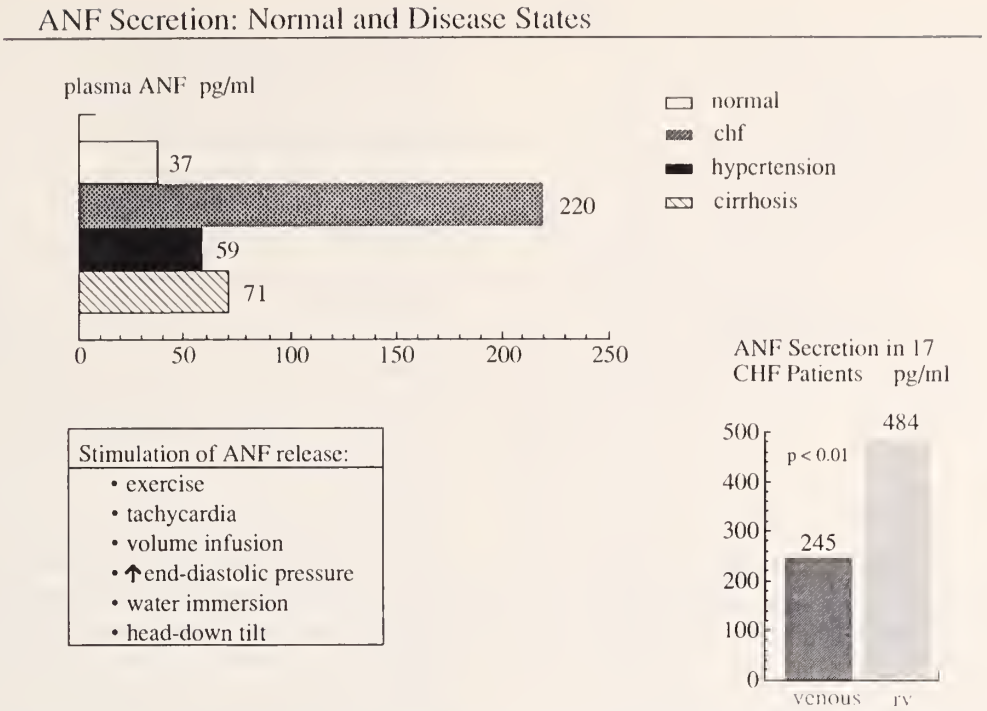


Fig. 4. Range of atrial natriuretic factor (ANF) levels in heart failure (CHF) compared with normal subjects and those with hypertension and cirrhosis. Stimuli for ANF release (box left) and evidence of myocardial production (box right). *Adapted* from Cody RJ. Atrial natriuretic peptide and the endocrine function of the heart. Primary Cardiology 1991; 17:14–28.

within cells? This is where some of the most exciting ideas will arise in this upcoming decade.

Clinical Value of Blocking Renin System. In terms of clinical trials, the deservedly well-known results of the CONSENSUS trial (13) show the favorable effect of enalapril over placebo. Key in this study was that it was the first major study of any drug therapy in heart failure which demonstrates a favorable effect on survival within the treatment group. And this fueled much of the work that has followed since 1987. The SOLVD study (14), published in the summer of 1991, has now confirmed that enalapril has a favorable effect on survival when compared to placebo. Similar conclusions were drawn from the Veterans Affairs study published in the same issue of *The New England Journal of Medicine* (15), showing that survival was greater with enalapril than with hydralazine and isosorbide dinitrate. The greater benefits of enalapril were significant after two years, but I think it is clear that the differences diminish at five to six years. That should not be a surprise, because the 5–6-year point is where the underlying high mortality rate in these patients probably overcomes any kind of therapeutic pharmacologic effect. In addition, the authors of the latter study suggested that these drugs might be used together, since they appear to have independent beneficial effects.

Clinical Value of Atrial Natriuretic Factor Enhancement? It is clear, then, that some judgment can be made on the clinical value of blocking the renin system. How about atrial natriuretic factor? The stimuli for ANF release are several (Fig. 4): exercise, tachycardia, volume infusion, and increased end-diastolic pressure in the ventricle. If one performs head-out water immersion, head-down tilt, ANF will go up. Any such maneuvers increase atrial tension, which at this time remains the primary mechanism for ANF release. The fact that ANF is released from the myocardium is documented by comparing plasma venous levels of ANF with levels simultaneously acquired in the right ventricle. Since ANF is produced by the myocardium, it will be carried into the coronary sinus, and the right ventricle is going to be the first mixing chamber for the ANF step-up.

In a group of patients with heart failure, it was clear that the ANF step-up at the right ventricular level is consistent with myocardial production (16). When these and similar results were identified in the early 80s, many groups, including our own at Cornell, pursued the finding vigorously. At first, it appeared that ANF would probably have the greatest celebrity and the quickest death of all the neurohormones in congestive heart failure. I would call that early stage

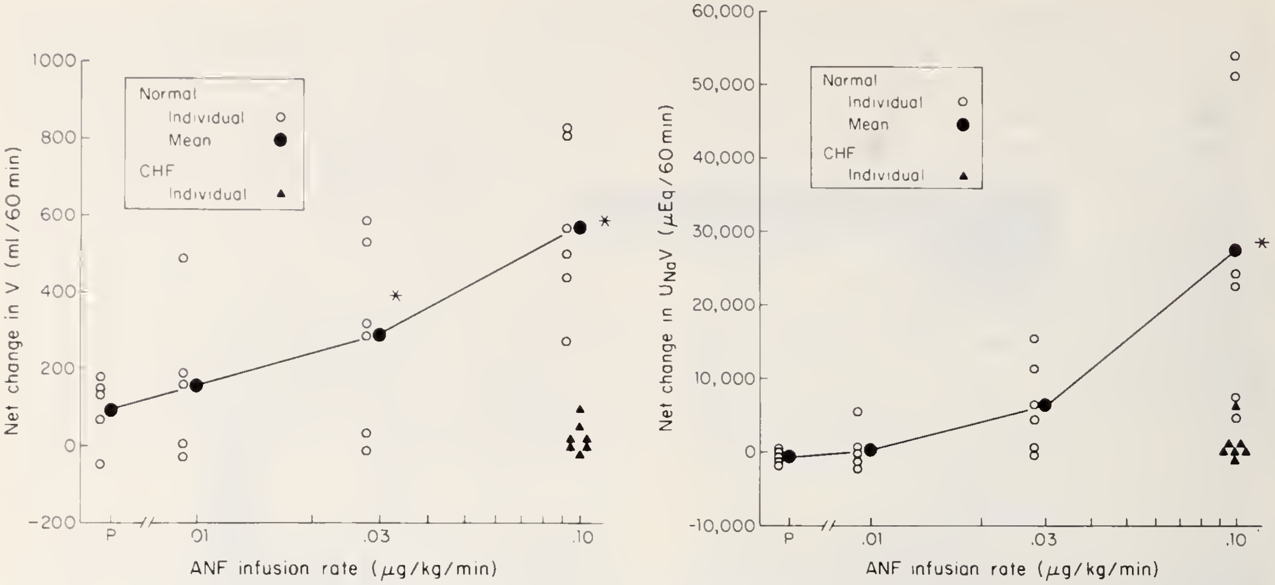


Fig. 5. Net change in urine volume (left) and sodium excretion (right) due to atrial natriuretic factor (ANF). Dose-dependent response occurred in normal subjects but little change occurred in subjects with congestive heart failure. *Reprinted* from ref. 17.

the dating stage of the relationship; now we are getting into the long-haul marital stage. I think much is still unknown about ANF. Keep in mind that ANF was identified only ten years ago, whereas the renin system was identified 45 years ago. ANF is not an adverse hormone, but one of the good guys. If it is a substance that stimulates vasodilation and diuresis, and it is clearly already produced in circulating levels much higher than normal, then how does one enhance its activity?

In one of the first passes we made at looking at ANF, we evaluated net change in urine volume and net change in sodium excretion (17) (Fig. 5). In normal subjects, urine volume and sodium excretion increase significantly with ANF infusion. Patients with congestive heart failure, particularly at the higher dose and even though they are sodium-overloaded, tend not to increase sodium excretion in a substantial way when compared with normal subjects. Perhaps sodium excretion increased a bit in one or two of the patients with heart failure. One of the problems in the literature is that many researchers express this small increase as a percentage change in sodium excretion, claim that sodium excretion is increasing 50% or 25%, and then examine the percentage change statistically, producing statistical significance. I think that a physician waiting at the bedside looking at the intake and output of his or her patient is not going to be impressed by such a small response after a couple of days, when many patients with heart failure have a baseline sodium secretion of less than 10 to 20 mEq per day.

So the clinical impact of ANF is currently not established. Perhaps we will learn more as we see the results of endopeptidase inhibition studies. Endopeptidase blocks endogenous ANF degradation, which might be important, particularly in mild heart failure, in augmenting sodium excretion and vasodilation. Additional information on other natriuretic substances such as urodilatin, and ANF receptor subtypes will also clarify issues.

New Insights and Understanding

The Renin System. There are several questions about the renin system which may provide new insight.

First is the issue of time dependence of renin system inhibition. Blockade of angiotensin II vasoconstriction with an ACE inhibitor is probably immediate and continues chronically. The effects of aldosterone might take days to weeks to be effective. Perhaps some of the most exciting information is on long-term benefits. Are the clinical benefits we observe simply the result of blocking angiotensin II, or are they the effects of angiotensin II on growth substances? Are these effects of angiotensin II on vascular smooth muscle hypertrophy? The data is now there. I believe that 4 or 5 years from now, we will be able to answer these questions more clearly.

One way to get a handle on some of the angiotensin-II-dependent versus angiotensin-II-independent effects of converting enzymes would be via some of the newer blockers. We now have renin inhibitors, actually the fulfillment of a quest of at least 15 or 16 years, when the first inhibitors were studied (18). We now have really effective angiotensin II antagonists, the fulfillment of a 20-year-old quest that began when the first intravenous angiotensin II antagonists were used and discarded because of their nonspecificity and combined agonist-antagonist effects. Some preliminary data from our laboratory on the response of blood pressure and hemodynamics to one of the newer angiotensin II antagonists, DUP753, in the spontaneously hypertensive rat demonstrate a favorable reduction of blood pressure associated with an increase in peak aortic blood flow and reduction of systemic resistance (19). This is one of the new approaches that will more clearly give us a handle on some of the effects of ACE inhibitors that are truly dependent on angiotensin II.

Endothelial-Based Vasoconstrictor Substances. Endothelial-based vasoconstrictor substances cannot be described as hormonal substances, at least not yet, but that may change in the future. These substances are endothelium-dependent, and they produce either vasoconstriction or vasodilation. Based on the juxtaposition of renin and ANF over the last few years, in my mind, any abnormality or pathophysiologic condition should be due to a vasoconstrictor and not a vasodilator. With this in mind, one may compare the endothelial vasodilator EDRF with the vasoconstrictor endothelin. At first glance, vasoconstriction produced by endothelin would seem to be clinically relevant.

In studies from our laboratory on the response of Sprague-Drawley rats to ET3, the rat amino acid sequence of endothelin, in 2 nmol/kg doses, produces a change of blood pressure from a mean baseline of 125; the initial response was hypertensive, with a mean to 70. Within a few minutes, the level returned to baseline and then was fixed at a hypertensive response (20). This is produced by changes in vascular tone, as changes in vascular resistance parallel blood pressure. That is all well and good when endothelin is given in pharmacologic doses, but can we get some insight into normal secretion of this peptide? Is it relevant in disease states?

A study published as a letter to the editor in 1989 showed that plasma concentrations of ET1, big ET1, the slightly larger amino acid sequence, were measured in acute myocardial infarction in normal subjects (21). These changes were compared to those in normal subjects; in myocardial infarction there was a significant increase of both big endothelin and ET1. Several studies have now demonstrated that ET1 is increased in conditions of ischemia.

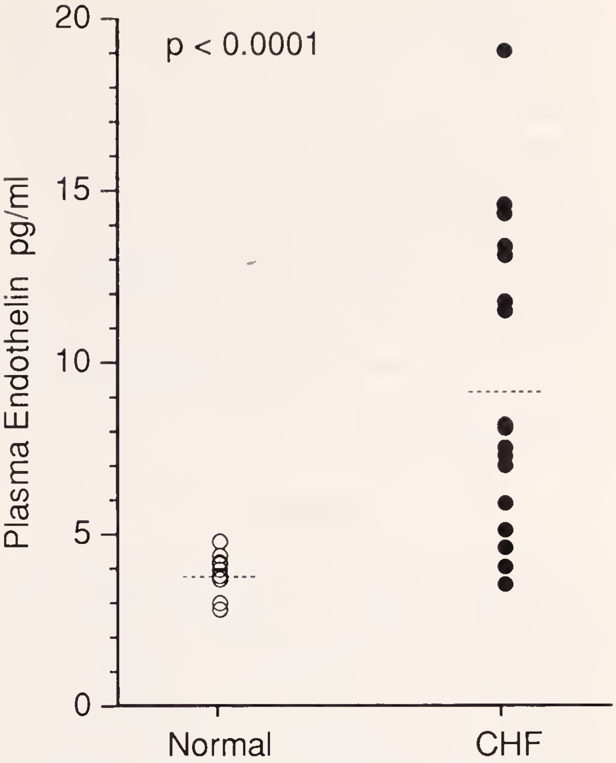


Fig. 6. Circulating immunoreactive endothelin in normal subjects and congestive heart failure patients. *Reprinted* from ref. 22.

New data from our laboratory (22) provides measurements of plasma endothelin in patients with congestive heart failure (Fig. 6). Results in normal subjects and those with heart failure demonstrate that we are coming back to the discovery stage. Endothelin is a substance we can measure in the circulation. My initial prejudice was that it was a substance with primary paracrine function—in other words, produced by the endothelium with primary effects on the nearby vascular smooth muscle. But why can we measure it in the circulation? Does this imply an endocrine role for it? Just as there is a wide range in renin values (Fig. 1), so there is also a wide range of endothelin values in patients with heart failure, at least a number of patients overlapping normal ranges.

Thus the question becomes, How do we explain this range and put it in a pathophysiologic context, with hemodynamic characteristics? I was not expecting to find a significant clinical correlate, but when we did the analysis, I was literally floored by observing a strong correlation between endothelin levels and pulmonary hemodynamics; the higher the endothelin level, the higher the pulmonary pressures, whether they were systolic, mean, or diastolic. The greatest correlation, with an r value of 0.9, was a correlation of these values with the resistance index.

Therefore, when other things are pretty much equal, the strongest correlate of increased endothelin was in the patients with the highest pulmonary pressures, one of the toughest groups of heart failure patients to manage. The patients with the highest pulmonary pressures cannot get onto our transplant waiting list. They are patients who tend to have a poor response to vasodilators. Other studies support this observation. Stewart and colleagues looked at endothelin levels in primary and secondary pulmonary hypertension, and demonstrated endothelin levels were higher in subjects with these disorders than in normal subjects (23).

Summary

Thus, we have learned a good deal about neurohormonal substances. I have summarized these neurohormonal data as the discovery stage, factors that modulate neurohormonal activity, identification of surrogates for this activity, the clinical impact of neurohormonal activity, and newer insights and understanding on these neurohormonal factors. In some ways we are gaining newer insights, but in other ways we find ourselves back in the discovery stage again.

Questions and Answers

Gorlin: Let me ask you a couple of questions if I may. But first, you alluded to the disappointing effects of ANF. Does any of this relate to some of the things that Burnett (24) has reported, that in congestive heart failure there is a production of defective ANF, a beta ANF, without the same kidney effects? Do you think that the kind of hormone generated has something to do with its metabolic action? You may measure its level by an assay, but maybe it is not doing what it should be doing at the end organ.

Cody: Dr. Gorlin, I think if one looks at response to ANF in mild heart failure, generally one will find greater natriuresis and a vasodilator response. I think part of the problem is that, as in many other attempts at therapy in heart failure, the first group of patients sought out were those with the most severe heart failure conditions.

In terms of what ANF does in normal subjects and in those in early stages of pathologic disorders, it has its greatest favorable impact in the patients with milder heart failure. I think ANF helps prevent the progression from mild to severe heart failure for a long period. It is eventually overwhelmed. Since heart failure exists for a long period, perhaps (as for many other neurohormonal substances) what is produced by the myocardium, where it is produced, also become deranged. I think it is a combination of a disruptive targeted organ response, and probably a later development of abnormalities of production and the characteristics of the peptide itself.

Gorlin: Do you still feel that digitalis has inhibitory effects on some of these neurohormones? You produced some of the most important data in that regard.

Cody: Yes, I still do believe that to be a factor. You are referring to the studies we performed of the response, to acute administration of digitalis, of renin and aldosterone which demonstrated suppression of both factors (25). Subsequent studies have also confirmed this observation. I would like to see data from studies of long-term administration, but unfortunately we never performed those studies. The results of the acute-administration studies and the information that has come out of the Digoxin-Captopril Multicenter Study (26) have convinced me that digoxin does something favorable, and I use it, particularly in severe heart failure patients, unless there is an absolute contraindication. Finally, Dr. Binkley and our group have been able to demonstrate changes in heart rate spectral analysis, consistent with decreased sympathetic activity.

Levitt: Dr. Cody, I enjoyed your presentation enormously. I was intrigued with some of the questions on endothelin with which you ended. But I have certain questions. You keep using the term “heart failure,” and you keep talking about its severity. I would like to understand how you measure the degree of heart failure, how you distinguish systolic failure from diastolic failure.

In respect to hyponatremia, apart from urine sodium excretion, I would prefer that you focus on thirst instead of ADH production, because thirst is what is necessary for hyponatremia.

Finally, I think you must be intrigued by the recent observations that atherosclerosis and ischemia destroy endothelial integrity and lead to enhanced plasma endothelin levels. In the current *New England Journal of Medicine* (27) there is a beautiful paper by Lerman and colleagues on this subject. Also, I am aware of studies in which renin release may be modulated by endothelin.

Cody: Thank you very much for your comments. I will try to respond in order. I did not preface my final remarks in terms of the patient population. These are patients with chronic congestive heart failure. There probably is important diastolic dysfunction, particularly in the coronary disease and hypertension subgroups. But I still think that at this time we are not separating systolic from diastolic pump failure as well, perhaps, as we should. That has to be addressed in the next few years.

I think generally I identify severity based on the New York Heart Association classification, which we are still using for clinical trials. I think the New York Heart Association classification, particularly in large studies, is still a reasonable way to classify the severity of heart failure.

The point you raise about hypoxia is an excellent one. One of my current thoughts is that endothelin might be a marker of hypoxia, because it is clear that during ischemia or even percutaneous coronary angioplasty, increases in endothelin occur. A recent study has demonstrated that endothelin goes up before CK goes up in the setting of myocardial infarction (28). So in some respects I think it is a marker of hypoxia. Now, maybe what's happening even in our heart failure patients and in pulmonary hypertensive patients is that myocardial ischemia may be greater with the higher pulmonary pressures. I think there is more to the issue than this, and we have to pursue such issues vigorously.

Your final point about endothelin and renin releases is an important one. I think it is probably particularly true in the kidney in terms of local modulation of renal function by renin and angiotensin II. A study in the *Journal of Clinical Investigation* in 1989 (29) reported that plasma renin activity goes up in response to endothelin. But acute administration of pharmacologic doses of endothelin may not translate into independent evidence of such physiologic interactions.

Kuhn: I think you made a good case for renin as a marker of severity of heart failure. In the rough clinical sense, apart from its physiologic connotations, do we need to measure plasma renin to determine the prognosis of the patient? In other words, can you glean the same prognostic information from the traditional clinical tests, perhaps an ejection fraction, or from the New York Heart Association classification? Is there added information you get when you have a positive renin?

Cody: Usually when we see a patient in functional class 4 failure, we basically know that many of these parameters and pathways are activated, even before we measure them. We are not surprised that many of these patients have hyponatremia or an increased serum creatinine and BUN, marked right-sided heart failure, and a low ejection fraction. All of these factors are associated with increased plasma renin activity. So perhaps New York Heart Association Class 4 patients become the ultimate surrogate for these markers. I have never really been impressed that ejection fraction as an isolated parameter is helpful.

References

1. Thomas JA, Marks BH. Plasma norepinephrine in congestive heart failure. Am J Cardiol 1978; 41:233–243.
2. Cody RJ, Franklin KW, Kluger J, Laragh JH. Sympathetic responsiveness and plasma norepinephrine during therapy of chronic congestive heart failure with captopril. Am J Med 1982; 72:791–797.
3. Cohn JN, Levine TB, Olivari MT, Francis GS, Rector S. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 1984; 311:819–823.
4. Cody RJ, Covit AB, Schaer GL, Laragh JH, Sealey JE, Feldschuh J. Sodium and water balance in chronic congestive heart failure. J Clin Invest 1986; 77:1441–1452.
5. Bayliss J, Norell M, Canepa-Anson R, Sutton G, Poole-Wilson P. Untreated heart failure: clinical and neuroendocrine effects of introducing diuretics. Br Heart J 1987; 57:17–22.
6. Kubo SH, Clark M, Laragh JH, Borer JS, Cody RJ. Identification of normal neurohormonal activity in mild congestive heart failure and the stimulating effect of upright posture and diuretics. Am J Cardiol 1987; 60: 1322–1328.
7. Francis GS, Benedict C, Johnstone DE, et al: SOLVD Investigators. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without heart failure. Circulation 1990; 82:1724–1729.
8. Brown JJ, Davies DL, Johnson VW, Lever AF, Robertson JIS. Renin relationships in congestive cardiac failure, treated and untreated. Am Heart J 1970; 80:329–342.
9. Packer M, Medina N, Yushak M. Correction of dilutional hyponatremia in severe chronic heart failure by converting enzyme inhibition. Ann Intern Med 1984; 100: 782–789.
10. Akselrod S, Gordon D, Shannon DC, Barger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science 1981; 213:220–222.
11. Binkley PF, Nunziata E, Haas GJ, Nelson SD, Cody RJ. Parasympathetic withdrawal is an integral component of autonomic imbalance in congestive heart failure: demonstration in human subjects and verification in a paced canine model of ventricular failure. J Am Coll Cardiol 1991; 18:464–472.
12. Cody RJ, Franklin KW, Kluger J, Laragh JH. Mechanisms governing the postural response, and baroreceptor abnormalities in chronic congestive heart failure: effects of acute and long-term converting enzyme inhibition. Circulation 1982; 66:135–141.
13. CONSENSUS Trial Study Group. Effects of enalapril on mortality in severe congestive heart failure. N Engl J Med 1987; 316:1429–1435.
14. SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med 1991; 325: 293–302.
15. Cohn JN, Johnson G, Ziesche S, et al. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. N Engl J Med 1991; 325:303–310.
16. Cody RJ, Atlas SA, Laragh JH. Physiological and pharmacological studies of atrial natriuretic factor, a natri-

uretic and vasoactive peptide. J Clin Pharmacol 1987; 27:927–936.

17. Cody RJ, Atlas SA, Laragh JH, et al. Atrial natriuretic factor in normal subjects and heart failure patients: plasma levels and renal, hormonal, and hemodynamic responses to peptide infusion. J Clin Invest 1986; 78: 1362–1374.
18. Cody RJ. Renin system inhibition: beginning the fourth epoch. Circulation 1992; 85:362–364.
19. Cody RJ, Haas GJ, Binkley PF, Brown DM. Hemodynamic and vascular characteristics of DUP 753: a specific angiotensin II antagonist in the spontaneously hypertensive rate (SHR). J Am Coll Cardiol 1991; 17: 202A.
20. Cody RJ, Haas GJ, Brown DM. Endothelin-induced hypertension is reversed by calcium channel antagonism in the rat. Clin Res 1990; 38:853A.
21. Miyauchi T, Yanagisawa M, Tomizawa T, et al. Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. Lancet 1989; 2:53–54.
22. Cody RJ, Haas GJ, Binkley PF, Capers Q, Kelley R. Plasma endothelin correlates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. Circulation 1992; 85:504–509.
23. Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? Ann Intern Med 1991; 114:464–469.
24. Wei CM, Kao P, Heublein D, Burnett JC Jr. The presence of an abnormal circulating form of atrial natriuretic factor in humans with congestive heart failure. J Am Coll Cardiol 1991; 17:316A.
25. Covit AB, Schaer GL, Sealey JE, Laragh JH, Cody RJ. Suppression of the renin-angiotensin system by intravenous digoxin in chronic congestive heart failure. Am J Med 1983; 75:445–447.
26. The Captopril-Digoxin Multicenter Research Group. Comparative effects of therapy with captopril and digoxin in patients with mild to moderate heart failure. JAMA 1988; 259:539–544.
27. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC, Jr. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. N Engl J Med 1991; 325:997–1001.
28. Stewart DJ, Kubac G, Costello KB, Cernacek P. Increased plasma endothelin-1 in the early hours of acute myocardial infarction. J Am Coll Cardiol 1991; 18:38–43.
29. Miller LW, Redfield MM, Burnett JC Jr. Integrated cardiac, renal, and endocrine action of endothelin. J Clin Invest 1989; 83:317–320.

Grand Rounds in Cardiology

Pathophysiology and Treatment of Hypertensive Heart Disease

Robert A. Phillips, M.D., Ph.D.

Left Ventricular Hypertrophy and Its Consequences

Left ventricular hypertrophy (LVH) occurs in upwards of 15–20% of adult hypertensives (1) and is a potent risk factor for future cardiovascular mortality. In a 10-year follow-up of 280 men and women with hypertension, those with baseline LVH (LV mass >125 g/m^2, the 95th percentile) had a two-fold greater incidence of morbid cardiovascular events (2), regardless of type of antihypertensive treatment. Although subjects with LVH have the worst prognosis, a relatively continuous relationship exists between cardiovascular risk and the range of LV mass. In the Framingham Heart Study, during four years of observation, each 50 g/m increase in LV mass was associated with a relative risk of cardiovascular disease of 1.49 for men and 1.57 for women (3). The effect on cardiovascular mortality was even more striking: a relative risk for each 50 g/m of 1.73 for men and 2.12 for women. LVH imparted an increased risk of sudden death. Whether regression of LV mass will lead to improved survival has not yet been conclusively determined, but preliminary evidence is encouraging (4). Hypertensive patients with LVH who experience a decrease in LV mass over a five-year treatment period appear to have a significantly lower incidence of cardiovascular events than those whose LV mass does not decrease.

Etiology. Since LVH is associated with significant morbidity and mortality, it is important to identify the factors which lead to this condition. Hemodynamic and nonhemodynamic stimuli for LVH have been identified.

Hemodynamic Factors. The relationship between systolic blood pressure and LV mass was first reported 60 years ago, and numerous subsequent studies have shown that arterial hypertension and consequent elevated wall stress is one important stimulus for LVH. The relationship between arterial pressure and hypertrophy can be explained by the Law of Laplace (wall stress $= P \times R/h$, where P is transmural pressure, R is the radius of the chamber, and h is the thickness of the wall). Elevated wall stress is a hemodynamic burden which reduces myocardial systolic performance. Since wall stress can be reduced by increased wall thickness, myocardial hypertrophy can be viewed as an adaptive cardiac compensation in response to chronically elevated blood pressure (5, 6). The role of blood pressure in development of hypertrophy is further supported by meta-analysis of 109 studies indicating that degree of blood pressure reduction accounts for approximately 25% of the variance of LV mass regression observed with treatment (7).

The sequence of events which leads from increased wall stress to cellular hypertrophy is under intense investigation. Increased stress most likely leads to activation of a stretch receptor, which then activates a series of growth genes that regulate myocardial cell protein synthesis.

Nonhemodynamic Factors. While increased wall stress is certainly an important signal for myocardial hypertrophy, clinical studies in hy-

---

Adapted from the author's Grand Rounds in Medicine presentation at the Mount Sinai School of Medicine in January 1990, revised and updated July 1992. From the Division of Cardiology, Department of Medicine, Mount Sinai School of Medicine. Address correspondence and reprint requests to the author, Director, Hypertension Section, Associate Director, Cardiovascular Training Program, Division of Cardiology, Mount Sinai School of Medicine, Box 1085, One Gustave L. Levy Place, New York, NY 10029.

pertensive patients show that for the same level of blood pressure, individuals vary widely in both prevalence and severity of LVH and vascular damage. Careful hemodynamic studies have indicated that only about 40% of the variation in LV mass in hypertensives is accounted for by differences in blood pressure, wall stress, and wall force (8). This has led to speculation that hormonal or other nonhemodynamic factors may play a role in the pathogenesis of hypertensive LVH (9). Some of the hormonal factors are chronic activation of the sympathetic nervous system, the renin angiotensin system (10), and presence of insulin resistance (11). Dietary factors such as alcohol (12) and sodium intake (13), as well as obesity (14, 15), may influence mass independently of their effect on blood pressure, as may whole blood viscosity (16). Therefore, prevention of LVH and optimal regression of LV mass may require modulation of these nonhemodynamic factors.

Race may also play a role in the development of LV structure and hypertrophy. In normotensive populations matched for blood pressure, African-Americans and whites have similar LV mass but the former have greater relative wall thickness and septal thickness (17). These early differences are amplified in a hypertensive population, where African-American subjects with similar elevations in blood pressure have greater ventricular mass than whites (18).

Physiological Consequences. Left ventricular hypertrophy is associated with multiple pathophysiological abnormalities which may account for the increased morbidity and mortality associated with the condition. These abnormalities include increased atherosclerosis and coronary perfusion abnormalities, such as reduced coronary flow reserve, altered coronary flow autoregulation, and subendocardial hypoperfusion, as well as abnormal myocardial pump function, such as LV diastolic dysfunction.

Abnormal Coronary Perfusion. One manifestation of abnormal perfusion, coronary flow reserve, can be assessed by measuring the difference between basal coronary flow and peak flow occurring in response to pharmacologically induced vasodilatory stress. In the resting state the hypertrophied heart has greater blood flow than the normal heart, but in response to stress, the ability of the hypertrophied heart to increase blood flow is significantly blunted (19, 20). When normalized per gram of tissue (mL/min/g), the hypertrophied heart has normal blood flow at rest, but may have a 50% reduction in blood flow in response to stress (21).

The reasons for reduced coronary flow reserve are complex and include structural and functional abnormalities of myocardial blood vessels such as reduced capillary density (22), reduced luminal diameter of intramyocardial small arteries (23), and increased vascular tone, possibly as a result factors such as abnormal endothelium-dependent relaxation (24). Hemodynamic factors may also play a role. The ability of LV filling pressures to decrease in response to stress is blunted in untreated patients with moderate or severe hypertension (25). Hence the fall in transmyocardial pressure gradient is blunted, and coronary vascular resistance remains high during stress (26).

Abnormal coronary flow reserve may be responsible for the reduced specificity with which thallium perfusion scan detects obstructive epicardial coronary disease in hypertensive patients. Although a negative thallium scan essentially rules out coronary disease in such patients (27), a positive scan may reflect abnormal coronary flow reserve rather than coronary stenosis in the hypertensive with hypertrophy (28).

Impaired coronary blood flow autoregulation is another manifestation of abnormal coronary perfusion in the hypertrophied heart. In experimentally induced LVH, while marked reductions in coronary perfusion pressure from 100 to 40 mm Hg have minimal effect on autoregulation in the subepicardium, the ability of the subendocardium to autoregulate is reduced by over 50% (29). This may account for the increased size of myocardial infarction associated with experimentally induced coronary ligation in hypertrophied hearts (30).

In human hypertensive patients without hypertrophy, coronary blood flow is maintained at a constant rate as perfusion pressure is acutely reduced with nitroprusside from 120 to 70 mm Hg. However, in hypertensive patients with hypertrophy a marked decline in flow occurs as perfusion pressure is reduced from 90 to 70 mm Hg (31). Thus, at least theoretically, reduction of blood pressure below 90 mm Hg in patients with LVH could cause ischemia. This may account for the controversial suggestion that a *J* curve describes the relationship between mortality rate from myocardial infarction and treated diastolic blood pressure in hypertensives with coronary artery disease: although reduction in diastolic pressure from 90 mm Hg to 85–90 mm Hg is associated with a twofold decrease in mortality, further blood pressure reduction may lead to increased death (32–35).

Animal studies suggest that abnormalities of coronary perfusion associated with hypertension and LVH are reversible. Reduction of blood pressure itself may improve coronary flow reserve, but normalization may require LV mass regression (36, 37).

Diastolic Function in Early Hypertension

Diastolic Abnormalities and Hypertrophy. Abnormalities of LV diastolic function are more common in the hypertrophied than the normal ventricle (38–40); they can manifest as clinically significant congestive heart failure (41), prolonged isovolumic relaxation (42), abnormalities of LV filling (43, 44), or increased cardiac filling pressures in response to ischemic stress (45). Diastolic abnormalities may be due to slower inactivation of the actin myosin complex as a result of such factors as reduced rate of sarcoplasmic uptake of calcium.

Abnormalities in Early Hypertension. Diastolic abnormalities may occur early in the course of hypertension and precede detectable hypertrophy (39, 40, 43, 44, 46–50). Young hypertensive persons demonstrate a tendency toward abnormal LV filling even before a detectable rise in blood pressure, suggesting that the factors which lead to these abnormalities may be inherited (51).

The clinical significance of diastolic abnormalities in early hypertension is not clear. Although a diastolic abnormality in early hypertensive heart disease is almost uniformly asymptomatic, it may indicate that the heart is beginning to hypertrophy in response to hemodynamic or nonhemodynamic stimuli (52). This is suggested by experimental evidence (in dogs) that as LV mass begins to increase in response to elevated pressure (induced by renal artery stenosis), LV filling becomes progressively more abnormal.

Modes of Evaluation. Diastolic function can be evaluated by several methods. The rate of isovolumic pressure decay, early and late LV filling, and pressure-volume relations can be derived from cardiac catheterization (53). Although these measurements are the most accurate indexes of diastolic function, they require an invasive procedure. Inferences regarding the diastolic properties of the ventricle can be obtained noninvasively by evaluation of LV filling.

Doppler Echocardiographic Evaluation. With certain caveats, Doppler echocardiography can be used to evaluate LV diastolic function in hypertensive persons. In the setting of normal ventricular relaxation, immediately after mitral valve opening, LV pressure is significantly lower than left atrial (LA) pressure; therefore the gradient between LA and LV is relatively high. This results in a high peak velocity of early filling (E) and significant emptying of the blood in the left atrium in early diastole. As a result, the late filling wave (A) is low. If LV relaxation is prolonged, LV pressure decline after mitral valve opening is delayed, so that the gradient between the left atrium and left ventricle in early diastole is reduced, and equilibration of pressure between the two chambers may be delayed. (Fig. 1). In the setting of normal LV function this is reflected on the Doppler recording as a reduced E and higher A/E ratio and/or a prolonged deceleration of the early filling wave (54, 55). One mechanism for the enhanced A wave is that as a result of decreased LV filling, LV pressure is probably lower than normal just prior to atrial contraction, and the delayed emptying causes a rise in atrial pressure. This combination leads to a higher gradient between the left atrium and left ventricle at atrial systole, and hence an enhanced peak A wave.

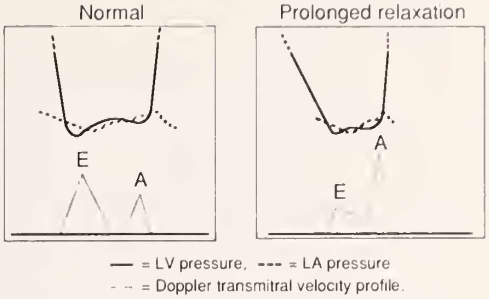
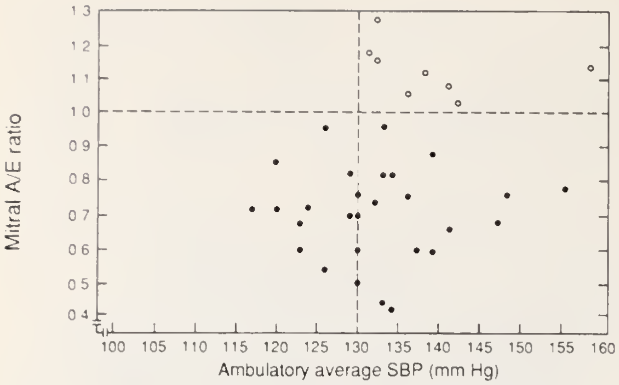

Normal
Prolonged relaxation
E
A
A
E
— = LV pressure, --- = LA pressure
- - = Doppler transmitral velocity profile.

Fig. 1. Left ventricular and left atrial pressure contour and corresponding Doppler transmitral velocity profile in normal subjects (left) and in states of prolonged left ventricular relaxation (right). *Adapted* with permission from ref. 87.

Application of this technique to the study of diastolic properties in humans must be viewed cautiously, since these variables can be affected by several factors, such as afterload on the ventricle (56), heart rate (57), LV chamber compliance, and systolic function (55). In addition, changes in left atrial pressure because of volume shifts can significantly alter these variables (54, 58).

Nevertheless, Phillips et al. demonstrated that normal subjects with heart rates ≤ 90 who are free of coronary disease and between the ages of 20 and 50 (mean 35 ± 9) have an average value for the A/E ratio of 0.67 ± 0.16; an A/E ratio of 0.99 is 2 SD above this mean value (44). This data has been corroborated by others who have similarly shown that a peak A to peak E ratio (A/E



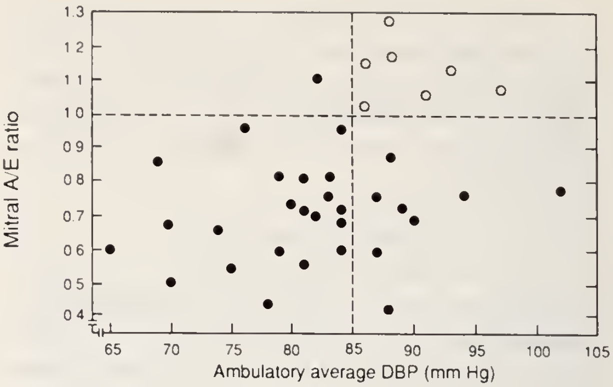


Fig. 2. **Left** Ratio of late to early left ventricular inflow flow velocity (A/E ratio) in 37 subjects plotted against average ambulatory systolic pressure. Horizontal hatched line indicates A/E ratio ≥ 1 is abnormal; vertical hatched line indicates blood pressure (130 mm Hg) above which abnormal A/E ratio was detected (eight subjects, open circles). *Adapted* with permission from ref. 44. **Fig. 3.** **Right** Ratio of late to early left ventricular inflow flow velocity (A/E ratio) in 37 subjects plotted against average ambulatory diastolic pressure. Only 1 of 22 subjects (closed circles) with diastolic pressure <85 mm Hg had abnormal A/E ratio. Hatched lines as in Fig. 2. *Adapted* with permission from ref. 44.

ratio ≥ 1 in subjects under 50 years of age) is significantly higher than the range for normal subjects (59). Therefore, an A/E ratio ≥ 1 in a hypertensive person with heart rate ≤ 90 and age ≤ 50 years of age is probably abnormal.

Abnormal Diastolic Filling and Blood Pressure. Using this technique, Phillips et al. found that approximately 20% of persons with untreated borderline or mild hypertension demonstrate diastolic filling abnormalities in the absence of LVH (44), and this data has been confirmed by others (60). In addition, there may be a threshold of average awake ambulatory blood pressure, 130/85 mm Hg, below which neither diastolic abnormalities nor LVH is detected (Figs. 2, 3). Using gated blood pool scans to measure the peak rate of early LV filling, White et al. have similarly determined that suspected hypertensives (subjects with office blood pressure >140/90) with ambulatory awake blood pressure <130/80 mm Hg have LV mass and diastolic function similar to normotensives, whereas suspected hypertensives with average awake blood pressure >140/90 have abnormal filling and increased LV mass (61). Taken together, these two studies suggest that subjects suspected of having hypertension on the basis of casual blood pressure readings are unlikely to have hypertensive heart disease if the awake ambulatory systolic blood pressure is <130 and the diastolic pressure is ≤/80–85 mm Hg.

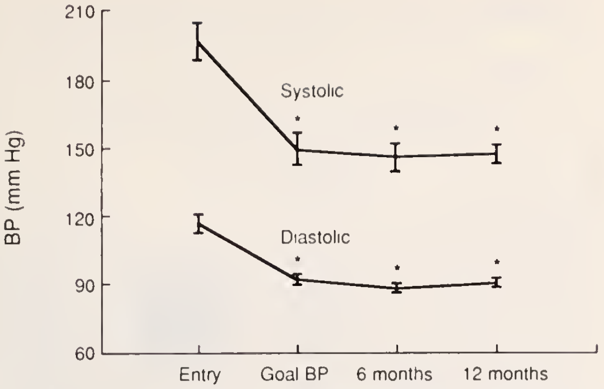
Effects of Therapy in Severe Hypertension

Nifedipine. If not treated, persons with severe hypertension have nearly 20% mortality within two years (62). Furthermore, LVH and its consequences are more prevalent in this group, and LV diastolic abnormalities tend to increase with hypertrophy (38–40). An extended-release formulation of nifedipine (nifedipine = GITS [gastrointestinal therapeutic system]) with pharmacokinetic properties suitable for once-daily administration (63) effectively lowers blood pressure in a sustained fashion in patients with severe hypertension (64). The ability of nifedipine-GITS, either as monotherapy or in combination with diuretics, to regress LVH was therefore studied in persons with hypertension (65).

Sixteen patients with initial diastolic blood pressure ≥ 120 mm Hg were treated for one year with nifedipine-GITS. Since changes in LV mass have not been unequivocally shown to have physiological benefit, associated alterations in LV systolic function, LV filling, plasma renin activity (PRA), atrial natriuretic peptide (ANP), and catecholamines were also evaluated.

Blood Pressure and Heart Rate. Blood pressure was significantly reduced from 200 ± 8/122 ± 3 mm Hg (mean ± SE) to 144 ± 5/89 ± 2 ($p <$ 0.0001) at one year (Fig. 4). Heart rate was 81 ± 5 bpm at entry and did not change over the course of the study (Fig. 2). Eleven patients (69%) required only nifedipine-GITS for blood pressure control; five (31%) required the addition of chlorthalidone. The median dose of nifedipine-GITS was 120 mg. Of the five patients who required chlorthalidone, four were taking 150 mg of nifedipine-GITS, and one was taking 90 mg.

Left Ventricular Mass. Left ventricular regression occurred over the course of six months and was sustained at one year (Fig. 5). This was a result of a decrease in wall thickness into the normal range without a change in LV chamber dimensions. Left ventricular mass regression was



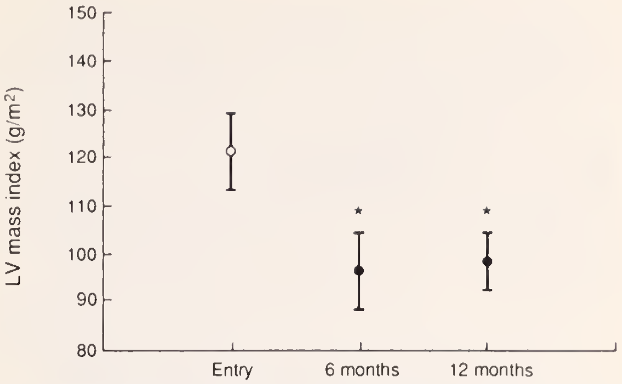


Fig. 4. Left Blood pressure in subjects with severe hypertension was significantly reduced by nifedipine-GITS monotherapy in 69% of subjects, and by combination of nifedipine-GITS with diuretic in remaining 31%. *Reprinted* with permission from ref. 65. **Fig. 5. Right** Effect of treatment on left ventricular mass. *p value <0.001. *Adapted* with permission from ref. 65.

confirmed by both echocardiographic and ECG criteria. The prevalence of echocardiographically determined LVH was reduced from 63% to 25% after one year of treatment (Fig. 6).

Several other studies have shown that calcium channel antagonists effectively regress LV mass (66–68). However, to my knowledge, no other study of calcium channel antagonists has shown LV mass regression in a population whose initial diastolic blood pressure was ≥120 mm Hg. Furthermore, in the only other previous report of regression of LV mass in severe hypertension, 89% required combination therapy with cilazapril (a converting enzyme inhibitor) and hydrochlorothiazide, and only in 11% could blood pressure be controlled with monotherapy (69).

Left Ventricular Systolic Function. Therapy affected systolic cardiac function by producing significantly higher fractional shortening after one year. Furthermore, the relationship between fractional shortening and end-systolic stress did not change over the course of treatment. This suggests that LV mass regression and treatment with nifedipine-GITS did not adversely affect intrinsic LV contractility (Fig. 7), a finding in accord with previous human and animal studies in arterial hypertension in which intrinsic LV contractility has been maintained after LV mass regression (70–73). Cardiac output did not change from entry.

Left Ventricular Diastolic Function. Left ventricular filling parameters obtained at one year were compared to those at end-titration (goal blood pressure), at which point blood pressure had been lowered and stabilized. Over the year there was a trend toward an increase in the velocity of early filling (57 ± 3 vs. 63 ± 4, $p = 0.07$), a trend toward a decrease in late diastolic filling (82 ± 4 vs. 78 ± 4), and significant decrease in the ratio of late to early peak velocity of LV filling (1.52 ± 0.1 vs. 1.28 ± 0.07, $p < 0.05$). There was a trend toward a relationship between LV mass regression and a decrease in the ratio of late to early peak velocity of LV filling ($r = 0.52$, $p = 0.07$). There was no relationship between change in heart rate and change in any of the parameters of LV filling.

The trend toward a relationship between LV mass regression and a reduced ratio of late to early filling is in accord with recent studies with calcium channel antagonists, converting enzyme inhibitors, and beta blockers, in which LV mass regression as a result of decreased wall thickness has been accompanied by apparently improved LV filling (66, 68, 74–76). However, there may be direct beneficial effects of nifedipine on LV diastolic properties independent of LV mass regression (77).

Catecholamines, Plasma Renin, and Atrial Natriuretic Peptide. In the course of one year no detectable activation occurred in either the catecholamine or the renin system. However, as expected, the trend among those subjects who required chlorthalidone was toward an increase in

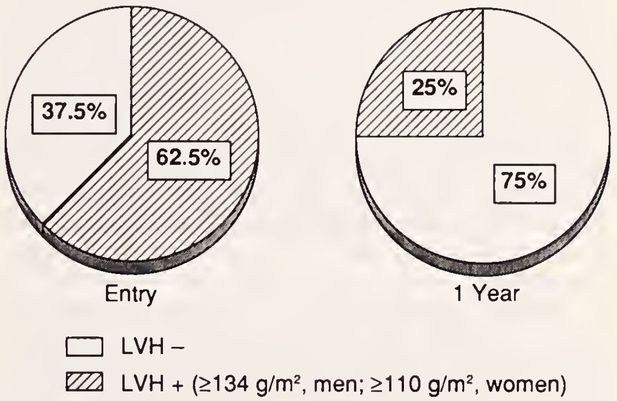


Fig. 6. Effect of treatment on prevalence of LVH. At 1 year, prevalence of LVH had decreased from 63% to 25%.

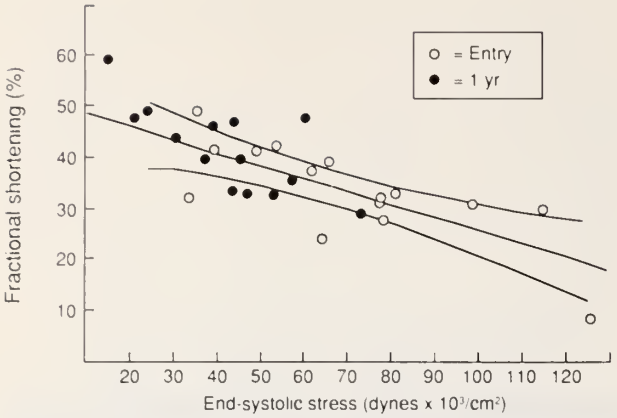


Fig. 7. LV contractility is preserved after LV mass regression with nifedipine-GITS. Plot of individual values for end-systolic stress (ESS) vs. fractional shortening (FS) before (open circles) and after (closed circles) 1 year of therapy, and of predicted regression line with 95% confidence intervals for the relation of ESS to FS. Relation did not change after LV mass regression, suggesting that intrinsic LV contractility was not altered. *Reprinted* with permission from ref. 65.

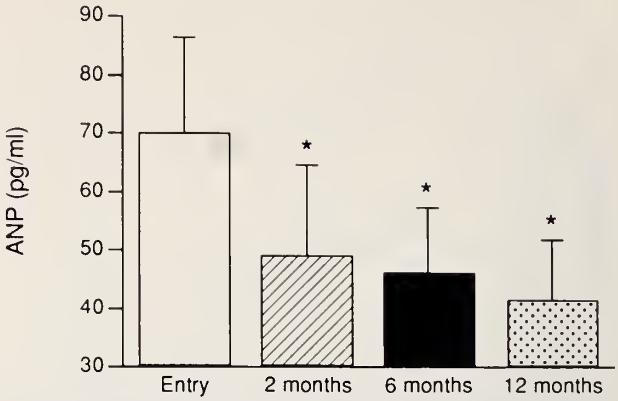


Fig. 8. Effect of 1 year of treatment with nifedipine-GITS on plasma atrial natriuretic peptide (ANP), which reflects cardiac filling pressures. Marked elevation at study entry reduced toward normal levels with treatment. *Adapted* with permission from ref. 65.

plasma renin activity (2.1 ± 0.9 vs. 5.6 ± 2.8 ng Angiotensin/mL/hr), whereas those treated with nifedipine-GITS monotherapy evidenced no change (1.3 ± 0.3 vs. 1.6 ± 0.4). Similarly, as expected, the trend among chlorthalidone-requiring subjects was toward an increase in plasma norepinephrine (374 ± 82 vs. 503 ± 122 pg/mL), whereas among subjects treated with extended-release nifedipine monotherapy the trend was toward a decrease (527 ± 77 vs. 459 ± 56). The stable catecholamine level in patients on monotherapy is probably a reflection of the pharmacologic profile of nifedipine-GITS: maintenance of a relatively constant level of the drug over 24 hours (63), leading to relatively minor fluctuations of blood pressure and hence little or no reflex sympathetic activation.

Atrial natriuretic peptide (ANP) was markedly reduced at two months, both in subjects on nifedipine-GITS monotherapy (64 ± 18 vs. 46 ± 11 pg/mL) and in those who required chlorthalidone (86 ± 27 vs. 41 ± 10). This effect was sustained at one year (Fig. 8). Since ANP inhibits renin secretion and possibly sympathetic system activation (78, 79), the decrease in ANP theoretically should have led to an increase in these two vasopressor systems. This did not occur. Thus, there was no neuroendocrine activation to offset the reduction in ANP and treatment with nifedipine-GITS.

While ANP has been shown to be elevated in hypertrophied hearts from hypertensive subjects (80), there was no relation between LV mass regression and reduction of ANP. Therefore, the most likely explanation for this finding is an improvement in cardiac filling pressure (81, 82). Atrial natriuretic peptide is secreted by the atria under conditions of increased atrial stretch (83). In the elderly person, the plasma level of ANP is significantly related to the risk of developing congestive heart failure (84). Although atrial natriuretic peptide is not elevated in all hypertensive patients, it appears to be elevated in severe hypertension (85), where cardiac filling pressures might increase due to elevated LV afterload (25). In this setting, release of atrial natriuretic peptide might be protective by virtue of its renal and systemic actions in reducing intravascular volume. With sustained control of arterial pressure, as occurred in this study, cardiac filling pressures and hence the stimulus for release of atrial natriuretic peptide would diminish. This is supported by the finding that, in untreated subjects with moderate and severe hypertension, exercise-induced increases in left atrial filling pressure is blunted after treatment with nicardipine, another dihydropyridine calcium channel blocker (25).

Side Effects. Trace peripheral edema was present in nearly 50% of subjects, but this did not lead to withdrawal of therapy. Gingival hyperplasia was found in two patients.

Clinical Implications. Blood pressure control in persons with severe hypertension has usually required multiple drug therapy with diuretics, peripheral adrenergic blockers, and vasodilators, with attendant side effects, cost, and difficulty in achieving patient compliance (86). This study demonstrates that treatment with nifedipine-GITS, in patients with diastolic blood pressure ⩾120 mm Hg, results in LV mass regression and effective control of blood pressure. Furthermore, this therapy is associated with favorable effects on LV performance and atrial natriuretic peptide, as well as a tolerable side-effect profile.

Left Ventricular Hypertrophy Current Strategy, Future Studies

Several important goals remain for the understanding and treatment of hypertensive heart disease. Prevention of hypertrophy, diastolic dysfunction, and coronary perfusion abnormalities is an obvious goal. Identifying the genes that mediate hypertrophy may allow design of agents that can turn off vascular and myocardial hypertrophy. Until these agents are available, conventional antihypertensive agents must be used. Meta-analysis of over 100 studies suggest that beta blockers, converting enzyme inhibitors, and calcium channel blockers are nearly equally effective in regressing LVH, whereas diuretics reduce chamber dimension but do not lead to regression of hypertrophied muscle (7). Until controlled trials of the efficacy of antihypertensive agents on LV mass regression emerge, the choice of drug may be dictated by the clinical setting, the efficacy with which an agent reduces blood pressure, and the presence of concomitant diseases which might dictate drug choice.

References

1. Levy D, Anderson KM, Savage DD, Kannel WB, Cristiansen JC, Castelli WP. Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors: the Framingham Heart Study. Ann Intern Med 1988; 108:7–13.
2. Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. Ann Intern Med 1991; 114:345–352.
3. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 1990; 322:1561–1566.
4. Koren MJ, Ulin RJ, Laragh JH, Devereux RB. Reduction in left ventricular mass during treatment of essential hypertension is associated with improved prognosis [Abstract]. Am J Hypertens 1991; 4:1A.
5. Grossman W, Jones D, McLaurin LP. Wall stress and patterns of hypertrophy in the human left ventricle. J Clin Invest 1975; 56(1):56–64.
6. Bader HS. Biological significance of cardiac hypertrophy. Am J Cardiol 1964; 14:133–137.
7. Dahlof B, Pennert K, Hansson L. Reversal of left ventricular hypertrophy in hypertensive patients. A metaanalysis of 109 treatment studies. Am J Hypertens 1992; 5:95–110.
8. Ganau A, Devereux RB, Pickering TG, et al. Relation of left ventricular hemodynamic load and contractile performance to left ventricular mass in hypertension. Circulation 1990; 81:25–36.
9. Frohlich ED, Tarazi RC. Is arterial pressure the sole factor responsible for hypertensive cardiac hypertrophy? Am J Cardiol 1979; 44:959–963.
10. Dahlof B. Factors involved in the pathogenesis of hypertensive cardiovascular hypertrophy: a review. Drugs 1988; 35(Suppl 5):6–26.
11. Phillips RA, Zimmerman S, Finegood DT, et al. Left ventricular mass is related to insulin resistance in mild hypertension [Abstract]. Am J Hypertens 1991; 4:14A.
12. Manolio TA, Levy D, Garrison RJ, Castelli WP, Kannel WB. Relation of alcohol intake to left ventricular mass: the Framingham Heart Study. J Am Coll Cardiol 1991; 17(3):717–721.
13. Schmeider RE, Messerli FH, Nunez BE, et al. Dietary salt intake: a determinant of cardiac involvement in essential hypertension [Abstract]. Am J Hypertens 1986; 4(Suppl 5):S556.
14. Lauer MS, Anderson KM, Levy D. Separate and joint influences of obesity and mild hypertension on left ventricular mass and geometry: the Framingham Heart Study. J Am Coll Cardiol 1992; 19(1):130–134.
15. Levy D, Anderson KM, Savage D, Kannel WB, Christiansen JC, Castelli WP. Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors. Ann Intern Med 1988; 108:7–13.
16. Devereux RB, Dravyer JIM, Chien S, et al. Whole blood viscosity as a determinant of cardiac hypertrophy in systemic hypertension. Am J Cardiol 1984; 54:592–595.
17. Hinderliter AL, Light KC, Willis PW. Racial differences in left ventricular structure in healthy young adults. Am J Cardiol 1992; 69:1196–1199.
18. Dunn FG, Oigman W, Sungard-Rise K, et al. Racial differences in cardiac adaptation to essential hypertension determined by echocardiographic indexes. J Am Coll Cardiol 1983; 5:1348–1351.
19. Pichard AD, Gorlin R, Smith H, et al. Coronary flow studies in patients with left ventricular hypertrophy of the hypertensive type. Evidence for an impaired coronary vascular reserve. Am J Cardiol 1981; 476:547.
20. Goldstein RA, Haynie M. Limited myocardial perfusion reserve in patients with left ventricular hypertrophy. J Nucl Med 1990; 31:255–258.
21. Hoffman JI. A critical view of coronary reserve. Circulation 1987; 75 (Suppl I):I-6–I-11.
22. Marcus ML, Harrison DG, Chilian WM, et al. Alterations in the coronary circulation in hypertrophied ventricles. Circulation 1987; 75 (Suppl I):I-19–I-25.
23. Tanaka M, Fujiwara H, Onodera T, et al. Quantitative analysis of narrowings of intramyocardial small arteries in normal hearts, hypertensive hearts, and hearts with hypertrophic cardiomyopathy. Circulation 1987; 75 (6):1130–1139.
24. Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990; 323:22–27.
25. Cody RJ, Kubo SH, Ryman KS, et al. Systemic and pulmonary hemodynamic responses to nicardipine during graded ergometric exercise in patients with moderate to severe hypertension. J Am Coll Cardiol 1987; 10:647–655.
26. Hoffman JIE. Determinants and prediction of transmural myocardial perfusion. Circulation 1978; 58:381–390.
27. Prisant LM, von Dohlen TW, Houghton JL, Carr AA, Frank MJ. A negative thallium (± dipyridamole) stress test excludes significant obstructive epicardial coronary artery disease in hypertensive patients. Am J Hypertens 1992; 5:71–75.

28. Houghton JL, Frank MJ, Carr AA, von Dohlen TW, Prisant M. Relations among impaired coronary flow reserve, left ventricular hypertrophy and thallium perfusion defects in hypertensive patients without obstructive coronary artery disease. J Am Coll Cardiol 1990; 15:43–51.
29. Harrison DG, Florentine MS, Brooks LA, Cooper SM, Marcus ML. The effect of hypertension and left ventricular hypertrophy on the lower range of coronary autoregulation. Circulation 1988; 77 (5):1108–1115.
30. Koyanagi S, Eastham CL, Harrison DG, Marcus ML. Increased size of myocardial infarction in dogs with chronic hypertension and left ventricular hypertrophy. Circ Res 1982; 50:55.
31. Polese A, DeCesare N, Montorsi P, et al. Upward shift of the lower range of coronary flow autoregulation in hypertensive patients with hypertrophy of the left ventricle. Circulation 1991; 83:845–853.
32. Cruickshank JM, Thorp JM, Zacharias FJ. Benefits and potential harm of lowering high blood pressure. Lancet 1987; 1:581–584.
33. Alderman MH, Ooi WL, Madhavan S, Cohen H. Treatment-induced blood pressure reduction and the risk of myocardial infarction. JAMA 1989; 262:920–924.
34. Farnett L, Mulrow CD, Linn WD, Lucey CR, Tuley MR. The J-curve phenomenon and the treatment of hypertension. Is there a point beyond which pressure reduction is dangerous? JAMA 1991; 265:489–495.
35. McCloskey LW, Psaty BM, Koepsell TD, Aagaard GN. Level of blood pressure and risk of myocardial infarction among treated hypertensive patients. Arch Intern Med 1992; 152:513–520.
36. Canby CA, Tomanek RJ. Role of lowering arterial pressure on maximal coronary flow with and without regression of cardiac hypertrophy. Am J Physiol 1989; 257:H1110–H1118.
37. Sato F, Isoyama S, Takishima T. Normalization of impaired coronary circulation in hypertrophied rat hearts. Hypertension 1990; 16:26–34.
38. Fouad FM, Slominiski JM, Tarazi RC. Left ventricular diastolic function in hypertension: relation to left ventricular mass and systolic function. J Am Coll Cardiol 1984; 3:1500–1506.
39. Smith VE, Schulman P, Karimeddini M, White WB, Meeran MK, Katz AM. Rapid left ventricular filling in left ventricular hypertrophy II. Pathological hypertrophy. J Am Coll Cardiol 1985; 5:869–874.
40. Inouye I, Massie B, Loge D, et al. Abnormal left ventricular filling: an early finding in mild to moderate systemic hypertension. Am J Cardiol 1984; 53:120–126.
41. Topol EJ, Traill TA, Fortuin NJ. Hypertensive hypertrophic cardiomyopathy of the elderly. N Engl J Med 1985; 312:277–283.
42. Hanrath P, Mathey DG, Siegert R, Bleifeld W. Left ventricular relaxation and filling pattern in different forms of left ventricular hypertrophy: an echocardiographic study. Am J Cardiol 1980; 45:15–23.
43. Phillips RA, Coplan NL, Krakoff LR, et al. Doppler echocardiographic analysis of left ventricular filling in treated hypertensive patients. J Am Coll Cardiol 1987; 9:317–322.
44. Phillips RA, Goldman ME, Ardeljan M, et al. Determinants of abnormal left ventricular filling in early hypertension. J Am Coll Cardiol 1989; 14:979–985.
45. Lorell BH, Grice WN, Apstein CS. Influence of hypertension with minimal hypertrophy on diastolic function during demand ischemia. Hypertension 1989; 13:361–370.
46. Hartford M, Wikstrand J, Wallentin I, Ljungman S, Wilhelmsen L, Berglund G. Diastolic function of the heart in untreated primary hypertension. Hypertension 1984; 6:329–338.
47. Shapiro LM, Mackinnon J, Beevers DG. Echocardiogaphic features of malignant hypertension. Br Heart J 1981; 46:374–379.
48. Papademetriou V, Gottdiener JS, Fletcher RD, Freis ED. Echocardiographic assessment of computer-assisted analysis of diastolic left ventricular function and hypertrophy in borderline or mild systemic hypertension. Am J Cardiol 1985; 56:546–550.
49. Dianzumba Sb, DiPette DJ, Cornman C, Weber E, Joyner CR. Left ventricular filling characteristics in mild untreated hypertension. Hypertension 1986; 8(Suppl I):I-156–I-160.
50. Fouad FM, Slominski JM, Tarazi RC. Left ventricular diastolic function in hypertension: relation to left ventricular mass and systolic function. J Am Coll Cardiol 1984; 3:1500–1506.
51. Graettinger WF, Neutel JM, Smith DHG, Weber MA. Left ventricular diastolic filling alterations in normotensive young adults with a family history of systemic hypertension. Am J Cardiol 1991; 68:51–56.
52. Douglas PS, Berko B, Lesh M, Reichek N. Alterations in diastolic function in response to progressive left ventricular hypertrophy. J Am Coll Cardiol 1989; 13:461–467.
53. Grossman W, McLaurin LP. Diastolic properties of the left ventricle. Ann Intern Med 1976; 84:316–326.
54. Choong CY, Abascal VM, Thomas JD, Guerrero JL, McGlew S, Weyman AE. Combined influence of ventricular loading and relaxation on the transmitral flow velocity profile in dogs measured by Doppler echocardiography. Circulation 1988; 78:672–683.
55. Himura Y, Kumada T, Kambayashi M, et al. Importance of left ventricular systolic function in the assessment of left ventricular diastolic function with Doppler transmitral flow velocity recording. J Am Coll Cardiol 1991; 18:753–760.
56. Brutsaert DL, Rademakers FE, Sys SU, Gillebert TC, Housmans PR. Analysis of relaxation in the evaluation of ventricular function of the heart. Prog Cardiovasc Dis 1985; 28:143–163.
57. Appleton CP, Carucci MJ, Henry CP, Olajos M. Influence of incremental changes in heart rate on mitral flow velocity: assessment in lightly sedated, conscious dogs. J Am Coll Cardiol 1991; 17:227–236.
58. Ishida Y, Meisner JS, Tsujioka K, et al. Left ventricular filling dynamics: influence of left ventricular relaxation and left atrial pressure. Circulation 1986; 74:187–196.
59. Van Dam I, Fast T, DeBoo J, et al. Normal diastolic filling patterns of the left ventricle. Eur Heart J 1988; 9:165–171.
60. Laufer E, Jennings GL, Dewar E. Prevalence of cardiac structural and functional abnormalities in untreated primary hypertension. Hypertension 1989; 13:151–162.
61. White WB, Schulman P, McCabe EJ, Dey HM. Average daily blood pressure, not office blood pressure, determines cardiac function in patients with hypertension. JAMA 1989; 261:873–877.
62. Veterans Administration Cooperative Study Group on Antihypertensive Agents. Effects of treatment on morbidity in hypertension: results in patients with diastolic

blood pressures averaging 115 through 129 mm Hg. JAMA 1967; 202:1028–1034.

63. Chung M, Reitburg DP, Gafney M, Singleton W. Clinical pharmacokinetics of nifedipine gastrointestinal therapeutic system: a controlled-release formulation of nifedipine. Am J Med 1987; 83(Suppl 6B):10–14.
64. Phillips RA, Ardeljan M, Goldman ME, Eison HB, Krakoff LR. Cardiac and hemodynamic adjustments to rapid and sustained blood pressure reduction. Am J Hypertens 1989; 2:196S–199S.
65. Phillips RA, Ardeljan M, Shimabukuro S, et al. Normalization of left ventricular mass and associated changes in neurohormones and atrial natriuretic peptide after one year of sustained nifedipine therapy for severe hypertension. J Am Coll Card 1991; 17:1595–1602.
66. Smith VE, White WB, Meeran MK, Karimeddini MK. Improved left ventricular filling accompanies reduced left ventricular mass during therapy of essential hypertension. J Am Coll Cardiol 1986; 8:1449–1454.
67. Szlachcic J, Tabau JF, Vollmer C, Massie BM. Effects of diltiazem on left ventricular mass and diastolic filling in mild to moderate hypertension. Am J Cardiol 1989; 63:198–201.
68. Schulman SP, Weiss JL, Becher LC, et al. The effects of antihypertensive therapy on left ventricular mass in elderly patients. N Engl J Med 1990; 322:1350–1356.
69. Sanchez RA, Trabelli CA, Marco EJ, Cianciulli T, Giannone CA, Ramirez AJ. Long-term evaluation of cilazapril in severe hypertension. Am J Med 1989; 87(Suppl 6B):565–603.
70. Grandi AM, Venco A, Barzizza F, Casadei B, Marchesi E, Finardi G. Effect of enalapril on left ventricular mass and performance in essential hypertension. Am J Cardiol 1989; 63:1093–1097.
71. Schmieder RE, Messerli FH, Sturgill D, Garavaglia GE, Nunez BD. Cardiac performance after reduction of myocardial hypertrophy. Am J Med 1989; 87:22–27.
72. Trimarco B, DeLuca N, Ricciardelli B. Cardiac function in systemic hypertension before and after reversal of left ventricular hypertrophy. Am J Cardiol 1988; 62:745–750.
73. Motz W, Strauer BE. Left ventricular function and collagen content after regression of hypertensive hypertrophy. Hypertension 1989; 13:43–50.
74. White WB, Schulman P, Karimeddini MK, Smith VE. Regression of left ventricular mass is accompanied by improvement in rapid left ventricular filling following antihypertensive therapy with metoprolol. Am Heart J 1989; 117:145–150.
75. Trimarco B, DeLuca N, Rosiello G. Improvement of diastolic function after reversal of left ventricular hypertrophy induced long-term antihypertensive treatment with tertatolol. Am J Cardiol 1989; 64:745–751.
76. Bielen EC, Fagard RH, Lijnen PJ, Tjandra-Maga TB, Verbesselt R, Amery AK. Comparison of the effects of isradipine and lisinopril on left ventricular structure and function in essential hypertension. Am J Cardiol 1992; 69:1200–1206.
77. Lorrell BH, Paulus WJ, Grossman W, Wynne J, Cohn P, Braunwald E. Improved diastolic function and systolic performance in hypertrophic cardiomyopathy after nifedipine. N Engl J Med 1980; 303:801–803.
78. Espiner EA, Richards AM. Atrial natriuretic peptide: an important factor in sodium and blood pressure regulation. Lancet 1989; 1:707–710.
79. Goetz KL. Physiology and pathophysiology of atrial peptides. Am J Physiol 1988; 254(Suppl E):E1–E15.
80. Takemura G, Fujiwara H, Mukoyama M, et al. Expression and distribution of atrial natriuretic peptide in human hypertrophic ventricle of hypertensive hearts and hearts with hypertrophic cardiomyopathy. Circulation 1991; 83:181–190.
81. Raine AEG, Erne P, Burgisser E, et al. Atrial natriuretic peptide and atrial pressure in patients with congestive heart failure. N Engl J Med 1986; 315:533–537.
82. Eison HB, Rosen MJ, Phillips RA, Krakoff LR. Determinants of atrial natriuretic factor in the adult respiratory distress syndrome. Chest 1988; 95:1040–1045.
83. Rodeheffer RJ, Tanaka I, Imada T, Hollister AS, Robertson D, Inagami T. Atrial pressure and secretion of atrial natriuretic factor into the human central circulation. J Am Coll Cardiol 1986; 8:18–26.
84. Davis KM, Fish LC, Elahi D, Clark BA, Minaker KL. Atrial natriuretic peptide levels in the prediction of congestive heart failure risk in frail elderly. JAMA 1992; 267:2625–2629.
85. Genest J, Larochelle P, Cusson JR, Gutkowska J, Cantin M. The atrial natriurectic factor in hypertension: state of the art lecture. Hypertension 1988; 11(Suppl I):3–7.
86. Mitchell HC, Pettinger WA. Long-term treatment of refractory hypertensive patients with minoxidil. JAMA 1978; 239:2131–2138.
87. Nishimura RA, Abel MD, Hatle LK, Tajik AJ. Assessment of diastolic function of the heart: background and current applications of Doppler echocardiography: II. Clinical studies. Mayo Clin Proc 1989; 64:181–204.

Grand Rounds in Cardiology

Management of Malignant Ventricular Tachyarrhythmias

J. Anthony Gomes, M.D., Stephen L. Winters, M.D., and Arisan Ergin, M.D.

In this discussion of the management of malignant ventricular tachyarrhythmias, I explore general concepts of management and our results at The Mount Sinai Hospital. Arisan Ergin then takes up surgical techniques in the management of ventricular arrhythmias, and Stephen Winters reviews our experience with the automatic implantable cardioverter defibrillator.

General Concepts

Malignant ventricular tachyarrhythmias usually occur as sustained symptomatic ventricular tachycardia, syncope, and sudden cardiac death. Syncope has a variety of causes. However, when syncope is the result of ventricular tachycardia, it is an independent predictor and precursor of sudden cardiac death. Of 235 patients studied over the last 5 years in our laboratory, 129 were referred for sustained symptomatic ventricular tachycardia, 60 were survivors of cardiac arrest, and 46 had syncope. The majority of these patients, about 75%, had coronary artery disease and significant left ventricular dysfunction. About 20% to 25% had cardiomyopathy and about 5% had no evident heart disease. About 75% of these patients had an ejection fraction of less than 40%; of these, about 70% had an ejection fraction of less than 30%.

Treatment modalities for patients with malignant ventricular arrhythmias have changed dramatically over the last decade. Whereas in the past only a few drugs were available, in the 1980s a variety of new drugs were approved by the Food and Drug Administration (FDA). In addition, other new modalities of therapy—such as surgery, devices, and catheter ablation—are currently available for the management of malignant ventricular arrhythmias.

Drugs. In the 1970s the only available agents were procainamide, quinidine, and norpace. Some of the drugs since approved by the FDA are good, and some have significant side effects. Procainamide, quinidine, and norpace are type 1A drugs that affect depolarization as well as repolarization of the ventricular myocardium. They are moderately effective for the management of these arrhythmias. Because norpace has a negative inotropic effect and can produce heart failure, we rarely, if ever, use it for the management of these patients. Subsequently, mexiletine and tocainide were approved. These are the type Ib drugs that affect depolarization but not repolarization. These drugs are at best only mildly effective in patients with malignant ventricular tachyarrhythmias. However, they are more effective used in combination with type 1a drugs than alone.

The other new drugs are flecainide, propafenone, and encainide, which were approved in the mid 1980s. Initially they were thought to be promising. They strongly affect depolarization and slow conduction in the ventricular myocardium. However, they do not increase refractoriness of the myocardium. Unfortunately, these

---

Adapted from the authors' Grand Rounds in Medicine presentation at Mount Sinai School of Medicine on October 16, 1990, revised June 1992. From the Electrophysiology and Electrocardiography Section, Division of Cardiology, Department of Medicine (JAG, SLW), and the Division of Cardiothoracic Surgery (AE), Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029. Address reprint requests to J. Anthony Gomes, Professor of Medicine and Director of Electrocardiography and Electrophysiology, Division of Cardiology, Box 1030, The Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

drugs have a potent negative inotropic effect and can induce heart failure. Also, they have a potent proarrhythmic effect in about 30% to 35% of patients with severe left ventricular dysfunction.

The drug sotalol is still experimental but is promising. It is a type III agent like amiodarone and also has beta blocker properties. Amiodarone clearly is the most efficacious drug available for the management of these patients. However, using this drug exacts a price: although the drug has minimal negative inotropic effects and hardly any proarrhythmic effects, it nonetheless has potent end-organ toxicity, particularly for such tissue as the lung and the liver. So if the patient is treated with amiodarone, he or she has to be closely followed up. That applies to all drugs; the patient so treated must be followed up carefully. More importantly, the physician has to know the side effects of each drug and watch for them carefully.

Beta adrenergic blockers and calcium channel blockers are used as adjunctive drugs for the management of these patients unless it is felt that sudden cardiac death was primarily ischemic in etiology. There is one kind of ventricular tachycardia, that due to catecholamines and induced by exercise, usually in the absence of ischemia, in which beta blockers and calcium channel blockers are used as prime modalities of therapy.

Surgery. When drugs fail to suppress arrhythmia, surgery is another option. In the past, aneurysmectomy was used in patients who had ventricular tachycardia, but soon it was realized that most of these patients had recurrences of ventricular tachycardia because that condition arises not from the aneurysm, which is electrically dead tissue, but most often from the border of or remote from the aneurysm. It has now been determined that the site of origin of ventricular tachycardia, particularly in patients with coronary artery disease, is the endocardium, and an operation involving surgical resection of the endocardium has been devised. This type of surgery is most often used in association with coronary bypass surgery. Clearly, endocardial resection can be done by other modes, such as cryoablation or freezing of the tissue at −60°C, and also by laser treatment of the tissue. This type of surgery carries a national mortality of about 10% to 15%.

Devices. The automatic implantable cardioverter defibrillator is a technological advance that has had a considerable impact on survival of these patients (see discussion by Stephen Winters below).

Another approach is that of catheter ablation. A catheter is placed in the ventricle and used to deliver electrical shock or radiofrequency current to ablate the tissue. This form of therapy will be the wave of the future, particularly for patients with surpraventricular tachycardias. It may turn out to be the treatment of choice for patients with right ventricular tachycardias associated with right ventricular cardiomyopathy and right ventricular dysplasia. When mapping techniques for the left ventricle are improved, it may be an option also for left ventricular tachycardia in association with coronary artery disease. However, the current success of catheter ablation for ventricular tachycardia is about 20%.

Clearly, the treatment of malignant ventricular arrhythmias has become superspecialized. In recognition of this development, the American Board of Internal Medicine in 1992 instituted the first board examinations for cardiac clinical electrophysiology.

Treatment modalities are selected by invasive testing known as electrophysiologic study. Malignant ventricular tachyarrhythmias are usually sporadic. Any episode of this arrhythmia might result in death. Therefore, the approach in the majority of patients is to induce the tachycardia in the laboratory by programmed electrical stimulation, and then terminate the tachycardia by the same method. Clearly, if the tachycardia results in hemodynamic compromise or degenerates into ventricular fibrillation, cardioversion is promptly carried out. Subsequently, a drug is given, usually orally, and the same method is used to determine whether by introduction of programmed extra stimuli one can induce or alter the characteristics of the tachycardia. If the tachycardia is suppressed, that is, one cannot induce it, therapy is deemed successful. If the tachycardia is inducible, the therapy is considered unsuccessful, particularly if the rate of tachycardia has not changed.

Obviously, this invasive procedure entails high risks. Nonetheless, when it is performed appropriately in a laboratory with trained personnel, the mortality rate approaches zero. In our laboratory, we have had no deaths in 207 consecutive patients with malignant ventricular arrhythmias as of October 1991, and the morbidity has been about 1%.

Prognosis. Potent determinants of survival in patients with ventricular tachycardia include the ejection fraction and the ease with which this arrhythmia can be induced and then suppressed by drug therapy. Wilber and co-workers (1) reported on survival among some 200 patients resuscitated after cardiac arrest. Survival curves were assessed for patients with inducible and suppressed tachycardia who were treated, in association with an ejection fraction, "good" equaling more than 30%, "low" being less than 30%. Patients who had a good ejection fraction and could be induced and suppressed had reasonably good survival, approaching about 92% over three years. Patients who had a low ejection fraction and could be induced and suppressed did not do as well; about 75% survived over a period of 3½ years. Patients who had a good ejection fraction and who were inducible but could not be suppressed by drugs or surgery had rather dismal survival rates, of about 40% over 3½ years. Patients who had a low ejection fraction and whose tachycardia could be induced but could not be suppressed did poorly. Their survival was only about 40% for a 1½ year period. Those who had a high ejection fraction and were not inducible in the baseline study did rather well, as did those patients who had a normal ejection fraction but whose tachycardia was inducible and suppressed. Those with tachycardia not inducible in the baseline study who had a low ejection fraction did not do as well; these patients had a survival rate of about 50% to 55% over two years. However, that observation in this group of patients remains highly controversial; in this study it accounted only for 8 patients. What is noteworthy is that inducibility and suppressibility by drugs or by surgery in association with a good ejection fraction is usually associated with a good prognosis. On the other hand, inducibility and nonsuppressability is associated with a bad outcome.

Therapy. How do we decide on the modalities of therapy for patients? I think that each institution, depending on experience, might approach this issue somewhat differently.

We have developed an algorithm on the basis of our experience over the last few years. All patients who have sustained ventricular tachycardia or fibrillation go to the coronary care unit or telemetry monitoring. They have the following tests: 24-hour Holter monitoring, a signal-averaging ECG to detect the presence of late potentials to determine the presence of an arrhythmic substrate, and a gated blood pool scan to assess ejection fraction; an ejection fraction less than 30% indicates the patient will probably not respond to any drugs except perhaps amiodarone. Patients who have survived an episode of sudden cardiac death should have coronary angiography. Treatment modalities would also be determined from the findings of coronary angiography as well as left ventricular geometry. Subsequent to that, the patient undergoes an electrophysiologic study.

If the patient has inducible sustained ventricular tachycardia or if the ventricular tachycardia degenerates into ventricular fibrillation, the following steps, primarily involving the use of drugs, surgery, or the automatic implantable cardioverter defibrillator (AICD), are taken.

Drug Treatment. About 60% of our patients are placed on drugs. The first-line drugs are usually type Ia, quinidine or procainamide in combination with mexiletine. Type Ic are rarely used in our laboratory because the majority of our patients have significant left ventricular dysfunction. If they are inducible on these drugs, they are considered for surgery, for amiodarone therapy, or for the automatic implantable cardioverter defibrillator with or without drugs.

If the ventricular tachycardia is significantly slowed and no longer associated with hemodynamic compromise, and the Holter monitor shows no high grades of ventricular ectopy, we might consider treating them with the drug which induces slowing of ventricular tachycardia.

Candidates for Surgery. There is a cohort of patients we believe are good candidates for surgery. They are patients who have a left ventricular anterior aneurysm and bypassable coronary artery disease. We consider surgery to be a better approach without a trial of drug therapy in these patients.

Also, other compelling factors, such as severe coronary artery disease, left main disease, left main equivalent disease, may compel the choice of surgery from the start, eliminating the drug algorithm. Eight to ten days after surgery we repeat the electrophysiologic study. Patients who are not inducible are discharged; those who are inducible receive an automatic implantable cardioverter defibrillator or amiodarone or other drugs.

Use of Cardioverter Defibrillation. A subgroup of patients we believe will do better with an automatic implantable cardioverter defibrillator (AICD) from the outset consists of those who have sarcoid and unpredictable ventricular tachycardia. For these patients AICD is the best protection.

In patients with other forms of cardiomyopathy, with sudden cardiac arrest, those with an ejection fraction of less than 30% in association with coronary artery disease, AICD may supplement drug therapy. An example: we had a 42-year-old patient recently who had syncope after consuming a moderate amount of alcohol while dancing at a wedding. The patient indeed had a history of cardiomyopathy and an ejection fraction of 20%. Three members of his family had died suddenly, his father at the age of 52 and two sisters at the age of 25 and 32. When brought to the laboratory the patient had sustained ventricular tachycardia induced at a rate of 240 beats/min with hemodynamic compromise. Clearly, the automatic implantable cardioverter defibrillator offered him the best protection. Thus, he did not go through the drug algorithm.

In patients who have survived ventricular tachycardia (VT) or sudden cardiac death who have stage III to IV heart failure and who are candidates for transplantation but not in status one for transplantation, the AICD may be considered a bridge to transplantation.

Variable Approaches. About 10% of patients with sustained VT, and about 30% to 35% of patients who have survived ventricular fibrillation (VF), may not be inducible. The treatment of these patients depends on the arrhythmia and the findings of coronary angiography. If we believe the ventricular fibrillation was due to ischemia, we advocate anti-ischemic therapy, and depending on the coronary angiogram, the patient may be considered for coronary bypass surgery. Not unusually, ventricular fibrillation is induced by antiarrhythmic drugs which are not really warranted, particularly for ventricular premature beats. In the laboratory for inducibility tests, when these patients are not taking such drugs they are not inducible; when they are taking such drugs tachycardia is inducible and all one has to do is to withdraw the drug. When hypokalemia and hypomagnesemia are suspected as the cause of VT/VF, these disorders should be corrected.

The therapeutic approach to patients who have an ejection fraction of less than 30% and are noninducible at EP testing remains controversial. Wilber et al. have shown that these patients do not do as well as those with higher ejection fractions. An automatic implantable cardioverter defibrillator, or amiodarone, should be considered, particularly if these patients have coronary artery disease or cardiomyopathy. Those who have primary ventricular fibrillation in the absence of overt heart disease, if not inducible, may be considered for an automatic implantable cardioverter defibrillator, amiodarone, or other forms of therapy.

In summary, then, the management of these patients is individually tailored. It is important to inform the patient of the therapeutic possibilities; some will not want surgery, some will not want drugs.

Treatment Choices, 235 Patients. Of about 235 patients studied in our laboratory, 59% were discharged on drug therapy, 16% had surgery, and 14% had an automatic implantable cardioverter defibrillator. No antiarrhythmic therapy was prescribed in 11%; these patients were primarily not inducible and had good ejection fractions.

The prime antiarrhythmic drug was amiodarone, either alone or in combination with other drugs; it was prescribed for 33% of the patient population on drug therapy. The second most common prescription among these patients (25%) was for type Ia or type Ib drugs alone or in combination. Sotalol, which is an experimental drug, was used to treat 8 patients. We rarely used type Ic drugs, which were popular in the mid 1980s, because we believed that these drugs had a high proarrhythmic profile, and therefore chose them for only 2% of our patients. These patients had good ventricular function.

Beta blockers were used alone in 8 patients, and these patients had exercise-induced VT. Beta (adrenergic) blockers as adjunctive therapy were used in 10 patients.

Surgery was performed on 35 patients; in 13 of these, VT had occurred 8–60 days after myocardial infarction and in 22 VT had occurred remotely after myocardial infarction. These were very sick patients; 23% had an ejection fraction of more than 30%, 77% had an ejection fraction of less than 30%, and 97% had an ejection fraction of less than 40%. The epicardial and endocardial surface of the left ventricle was mapped in the operating room in sinus rhythm and during induced ventricular tachycardia.

In these patients surgery was quite extensive. Of 35 patients, 29 had an endocardial resection; additionally, coronary bypass was performed in 26, aneurysmectomy in 18, cryoablation in 7, mitral valve replacement in 2, and automatic implantable cardioverter defibrillator patches were placed in 5 patients. Our operative mortality remains one of the lowest in the country in these high-risk patients: only 1 out of 35, or 3%. The endocardial resection was map guided in 26 patients (74%) and blind, that is, without mapping because of technical problems or because the patient's tachycardia was noninducible in the operating room, in 9 patients. On postoperative electrophysiology study, 89% were not and 11% were inducible. Of the inducible patients, one had an automatic implantable cardioverter defibrillator, one was treated with quinidine, and one with amiodarone. Spontaneous VT or sudden death occurred in 17%; in 25-month follow-up, sudden death had occurred in only 1 patient. The other 5 patients had sustained VT which was treated appropriately.

Patients with sustained VT or VF after late myocardial infarction are difficult to manage. They have recurrent recalcitrant VT, and usually do not respond to medical therapy. The VT usually occurs about 8 days to 6 weeks after myocardial infarction. The mean in our institution is 14 ± 4 days. These patients had a dismal long-term survival of less than 60% in the 1970s and 1980s. In the 1990s the survival improved to 85%–90%. In our own study the survival was 91% for 20 months (2). In the study of Willems et al. (3) reported recently, poor survival of 66% was noted for prophylactic empiric antiarrhythmic therapy. Surgery as well as devices were used late in the course of therapy. In contrast, in our study we used EP-guided antiarrhythmic therapy. If patients did not respond to two drugs and had recurrent episodes of VT, we immediately proceeded to surgery, and in my view, our excellent results were achieved because approximately 50% of our patients had endocardial resection in association with coronary bypass.

Survival. How does this aggressive management influence survival? From historical controls, it seems that patients treated with the automatic implantable cardioverter defibrillator have the best survival for sudden cardiac death; next best is EP-guided therapy, then VT surgery. Empiric therapy is the worst and is not used in this country.

How do our own results compare? Our follow-up in 235 patients for a mean of about 18 months showed the following. Of patients who were treated with drugs, the recurrence of VT or sudden cardiac death was 20%. Of patients who had surgery, the recurrence was 17%; of the patients who had automatic implantable cardioverter defibrillator, 44% have used the device for what we call true shocks. Therefore we believe that if these patients had not had the automatic implantable cardioverter defibrillator, they would either have died or had sustained VT, with its dire consequences. Sudden cardiac death occurred in 11% of patients treated with drugs, in only 3% of patients who had surgery, and in 3% of patients who had the automatic implantable cardioverter defibrillator. Clearly, the rates of survival for sudden cardiac death are better for patients treated by drugs and surgery than has been reported in other studies; this may be related to several factors, including the patient population and selection criteria.

The treatment of these patients is challenging; success or failure is dependent on a close collaboration between surgeons and electrophysiologists, and interaction between the nursing staff, psychiatrists, and psychologists for support of these patients.

I believe that we have generally been disappointed by drug therapy; an ideal antiarrhythmic drug is not available at this time. Basic scientists need to assess a new generation of drugs, not drugs that affect the entire heart on an ionic basis, but rather drugs selected for targeted ablation or modulation of the substrate. Perhaps advances in genetics will help in this regard. However, this approach may indeed take decades. Until then, improvement in mapping techniques and analysis will assure use of ablation techniques, and improvement in devices will be the wave of the future.

Finally, prevention is better than cure. I am proud to say that our laboratory has been in the forefront in utilizing risk stratification and application of strategies to prevent sudden cardiac death.

Surgical Treatment

ARISAN ERGIN

The surgical treatment of ventricular tachycardia was introduced in the late 1970s and early 1980s; after about a decade, the field is still in a state of evolution. Two main techniques are used for surgical ablation. One aims at isolating the active electrophysiologic substrate from the rest of the myocardium, and that is encircling endocardioventriculotomy. The total and blind endocardioventriculotomy has been mostly abandoned because of poor functional results. It basically destroys the function of the heart, producing a nonarrhythmic heart with severe left ventricular dysfunction. The partial guided endocardioventriculotomy was introduced as a compromise, and it has been increasingly used.

The technique we favor is endocardial resection. It is usually used in a guided and extended fashion. Intraoperative mapping localizes the substrate, and we aim at resecting the visible segment of the heart, including most of the scar. More recently, sequential guided endocardial resection has been introduced to deal with multiple origins of tachycardia. Cryoablation and laser ablation are used only as supplements to the main techniques of encircling endocardioventriculotomy or endocardial resection.

Case Report and Description of Technique. A patient was referred from another institution after a 10-day hospital course following an acute myocardial infarction. He had a stormy postinfarction course at that hospital, requiring several antiarrhythmic drugs and combinations thereof and had already been cardioverted on numerous occasions. On arrival at Mount Sinai, he had an ejection fraction of 13%. He was already tracheostomized and he had several burns on his chest and a broken sternum from resuscitation efforts. He was recommended for surgery because he had single-vessel disease and anterior wall infarction, as evidenced on his catheterization and drug-unresponsive sustained unstable VT. He had intraoperative mapping, an integral part of the surgical treatment. We use a ribbon electrode, initially mapping in sinus rhythm. This area of electrode usually localizes the abnormal substrate, and then ventricular tachycardia is induced, and the activation mapping is then carried out during ventricular tachycardia.

Once the epicardial mapping is satisfactory, we proceed to endocardial mapping. I believe the most important part of the procedure is to localize the substrate endocardially. To be able to do that, the patient is placed on cardiopulmonary bypass. The patient is still normothermic. The heart is beating and it is opened right through the infarct. Some stay sutures are then placed. At that point the patient is on total cardiopulmonary bypass, and there is pressure in the aorta even though the ventricle is open. No air will get up into the aorta, but there is always danger of air embolism when the heart is opened. Again, with the ribbon electrode the endocardial surface of the left ventricle is totally mapped, both in sinus rhythm and during ventricular tachycardia. Dr. Gomes at the back table looks at the squiggly lines and he tells me where the substrate is. I have to take his word for it because I do not really see it. And then once he is satisfied, he says, it is there, just go get it, and then we resect it. To stop the heart, the aorta is cross-clamped; we give cardioplegia to protect the rest of the ventricle. In this patient the origin of the tachycardia was localized to the septum. A plane is then developed between the scarred septum and the underlying myocardium, and the endocardium is then resected. In patients like this man, who came early after myocardial infarction to surgery, this plane is not well developed, so one has to be careful not to excise too much, otherwise the result is a hole in the septum. In this patient, the specimen that was removed was rather thick, because—not being able to tell where the plane actually was, and wanting not to miss the substrate in the process of taking too thin a slice—we excised most of the septum. This surgery aims at removing all the visible scar; even though I take Dr. Gomes' and Dr. Winters's word that it is there in the middle, we still go beyond where we think it really is to make sure everything is out.

We tend to use guided, extended endocardial resection rather than the localized endocardial resection, in which the surgeon depends on the guide and excises only a local area. When the specimen is removed, the septum is usually left quite thin. In this patient we elected to use a pericardial patch to reinforce the septum because of fear of ending up with a postoperative ventricular septal defect in this infarcted, partially attenuated septum. The specimen included the better portion of the septum. We then sutured a piece of autologous pericardium into the area to reinforce this denuded septum to prevent ventricular septal defect formation. The other way of doing this is on a beating heart, that is, sequential endocardial resection; in other words, mapping, resetting the area, reinducing tachycardia if it is again inducible, remapping, finding another area, resecting it. We believe this procedure of repeated mapping and endocardial resection imposes quite a burden on the heart.

The patient's pericardium is sutured and the ventricle closed. The ventriculotomy is then closed by taking sutures well away from the infarcted area because you cannot suture to dead muscle, which will not hold stitches. This procedure in fact will get rid of the arrhythmic substrate as well as most of the infarcted muscle and, it is hoped, will result in improvement in ventricular function.

If this patient had had a scar close to the papillary muscle, we could not have resected, because resection would impair mitral valve function. For such a patient we would use cryoablation, or laser ablation of that area. Cryoablation will kill all the active cells without destroying the matrix of the heart, so there would be no structural weakening.

The ventriculotomy is then closed and the heart is beating. Because of this patient's stormy preoperative course, even though we were confident we got the focus, we still elected to place the automatic implantable cardioverter defibrillator patches, to be on the safe side in the postoperative period. In fact he was not inducible postoperatively, and he has done well. His postoperative gated blood pool showed his ejection fraction to be up to 28%, compared to 13% preoperatively.

The Implantable Defibrillator

STEPHEN L. WINTERS

"There is serious question whether an indication can be spelled out for the use of an implanted standby defibrillator. . . . In fact, the implanted defibrillator system represents an imperfect solution in search of a plausible and practical application." So said a 1972 editorial by one of the most prominent practitioners in arrhythmia management (4), showing how wrong some people can be some of the time. In 1972 any thoughts of future use for an implantable cardioverter defibrillator were viewed skeptically. In our own series, follow-up data are available on 32 patients who have received such a device. (We performed an additional 2 implants in the past few weeks). The bulk of our patients have poor ventricular function as determined from left ventricular ejection fraction. Over half of the patients failed at least 3 attempts at suppression of their tachycardia with various antiarrhythmic agents. As it often turns out when people undergo implantation of such a device, almost half had concomitant cardiac surgery of one form or another.

Postimplantation follow-up data for close to two years is available. Consistent with overall world experience, close to half of these patients required antiarrhythmic drugs, predominantly to prevent unnecessary shocks from the device. We had two periimplant deaths. The first turned out to be a patient who had possible proarrhythmic effects from a combination of antiarrhythmic agents (amiodarone and flecainide) which he had been taking; however, he also had a defibrillator implanted with a defective resistor.

In the follow-up period, two patients have died of noncardiac causes and 44% of the patients have had discharges delivered by the device. In an additional 20% of patients, probable false discharges were delivered from the device, a percentage consonant with international experience.

Efficacy in Prolonging Survival. A recent report of Fogoros and colleagues (5) compared expected survival and actual survival in patients who have ejection fractions less than or greater than 30% (Table). The actual survival is significantly better than expected in patients who have an ejection fraction over and under 30% with the implanted device—for these patients, more than 30% better than expected.

TABLE

Projected and Actual Cumulative Survival by Left Ventricular Ejection Fraction (LVEF)

LVEF	No. of pts	Cumulative survival			
		Actual		Projected	
		1 Yr	3 Yr	1 Yr	3 Yr
<30%	40	94 ± 4%	67 ± 12%	36 ± 9%	6 ± 15%
>30%	79	99 ± 1%	96 ± 3%	69 ± 6%	46 ± 8%

Adapted from Fogoros RN et al., J Am Coll Cardiol 1990;16: 381 (ref. 5).

The indications for implantation of automatic cardioverter defibrillators are still changing, but include patients who have recurrent, sustained ventricular tachycardia, ventricular fibrillation, or sudden cardiac death despite all efforts at treating with EP-guided treatment; patients in whom effective suppression of tachycardia cannot be achieved; patients who have had sustained ventricular tachycardia or ventricular fibrillation that is not reproducible in the EP laboratory (especially if they have compromised ventricular function or infiltrative processes); patients who have persistent induction of ventricular tachycardia even though a surgical procedure such as endocardial resection was performed; and patients who have sustained ventricular tachycardia or ventricular fibrillation suppressed at EPS, but for whom the therapy does not prevent recurrence. This latter situation might exist, for example, in patients who have sarcoidosis and sustained ventricular tachycardia.

Before a patient receives a device, several considerations are weighed, particularly whether the tachycardia is reproducible or reversible, and whether there is a remediable cause. One also has to consider such issues as whether there is a concomitant illness that might shorten the patient's lifespan and thus make it not feasible to implant a costly device requiring the patient to undergo uncomfortable surgery. In addition, one must consider whether the ventricular tachycardia or ventricular fibrillation might be too frequent and thus cause the patient to undergo many shocks as well as deplete the energy from the device quickly; whether the device can successfully terminate the tachycardia without subsequent tachycardia developing immediately afterward; and whether the patient can deal with the device psychologically. In view of some of these considerations, we frequently call in a psychiatrist for a patient before implantation of an automatic cardioverter defibrillator.

The best known and still the only FDA-approved device is a bulky one implanted in the abdominal wall. A series of leads are tunneled subcutaneously and are positioned around the heart, usually through a midline sternotomy, a thoracotomy, or (rarely) a subcostal approach. When the device is implanted, a complicated testing scheme is employed in the operating room to make sure the leads are positioned in the best location to sense the tachycardia and to determine that VT is present, as well as to effectively deliver enough energy to cardiovert the heart.

The standard device can administer up to 4 or 5 shocks sequentially and will then stop. The energy consists of up to 700 volts delivered over a brief period. It is predominantly the rate of the tachycardia that triggers the device to charge up and deliver a shock. Many newer, more complex and sophisticated devices now in clinical trials will soon be released. They are multiprogrammable devices similar to pacemakers, which can be interrogated. They have standard features of a regular antibradycardiac pacemaker. They can also act to pace the heart out of tachycardia. Many of the newer devices can be used to perform electrophysiologic testing without requiring additional electrode catheters in the heart through veins. In the future they may even be able to deliver boluses of antiarrhythmic agents.

Still, the leads that are used even with these devices tend to require epicardial positioning and sternotomies or thoracotomies. Ongoing research studies are looking at purely transvenous leads such as are used with standard pacemakers.

Questions and Answers

Leslie Kuhn: In your case material, I didn't see any case of myocarditis. In the noncoronary group with serious arrhythmias, do you do biopsies for cryptic myocarditis? If you do, and if a biopsy is positive, do you treat that patient, or does this not come up?

Gomes: It comes up. I think that we recommend biopsies in people who have ventricular tachycardia, sudden cardiac death in the absence of coronary artery disease or in the absence of a true myopathic picture. Usually, few patients agree to go along with biopsies, and the readings on biopsies we have done have varied widely between The Mount Sinai Hospital, Columbia, and Stanford.

So basically, although we suggest biopsies, not many are performed because the result does not influence our treatment. Many patients who have ventricular tachycardia who have possible myocarditis do not have heart failure and are not candidates for transplantation. However, steroids or immunosuppressive therapy may be entertained in these patients. They come primarily with ventricular tachycardia, and I think what they have is probably patchy myocarditis somewhere in the ventricle that creates a substrate.

Kuhn: You haven't treated any as such?

Gomes: No, not with immunosuppressive therapy or steroids.

Kuhn: Dr. Winters, how close are you, do you think, to a nonthoracotomy lead system, if you will?

Winters: Initial clinical trials on a transvenous lead system were stopped due to technical problems. However, efforts are progressing well to achieve this goal.

Allen Unger: Do you almost always put patients on medication plus automatic implantable cardioverter defibrillator, especially low-dose amiodarone, which seems to be less toxic in low dose? How do you feel about this?

Winters: Antiarrhythmic drugs are frequently required to minimize the number of discharges from the device, especially false ones due to nonsustained ventricular tachycardia.

Gomes: That depends, I think, on the recurrence of the arrhythmia. If a patient has had only a rare episode, one episode of VT or of sudden cardiac death, you may not want to treat the patient with drugs and you may just implant the automatic implantable cardioverter defibrillator and leave it at that. But we have to control the tachycardia of patients who have recurrent episodes—otherwise the device will go off frequently. So we usually use whatever drug we have already tried, one that changed the characteristics of the patient's tachycardia, or else we might consider the use of low-dose amiodarone.

Unger: Dr. Ergin, you're doing a lot of septal resection. Do you almost always put in a patch?

Ergin: No. That depends on the amount and thickness of the septum that is removed.

References

1. Wilber DJ, Garan H, Finkelstein D, et al. Out-of-hospital cardiac: use of electrophysiologic listing in the prediction of long-term outcome. N Engl J Med 1988; 318:19–24.
2. Gomes JA, Winters SL, Ergin A, et al. The clinical, electrophysiologic determinants of treatment and survival of patients with sustained malignant ventricular tachyarrhythmias, late post-myocardial infarction. J Am Coll Cardiol 1991; 17:320–6.
3. Willems AR, Tijssen JAP, Van Capelle FJL, et al. Determinants of prognosis in symptomatic ventricular tachycardia or ventricular fibrillation late after myocardial infarction. J Am Coll Cardiol 1990; 16:521–530.
4. Lown B, Axelrod A. Implanted standby defibrillators. Circulation 1972; 46:637.
5. Fogoros RN, Elson J, Bonnet CA, Fiedler SB, et al. Efficacy of the automatic implantable cardioverter defibrillator in prolonging survival in patients with severe underlying cardiac disease. J Am Coll Cardiol 1990; 16: 381.

Grand Rounds

Graves' Disease and Its Retroorbital Aspects

Terry F. Davies, M.D., FRCP

For some 50 years, beginning after World War II, when the use of radioiodine was pioneered in this country by among others Quimby, Feitelberg, and Silver at The Mount Sinai Hospital (1), this institution has maintained an international reputation for contributions to the understanding and treatment of thyroid disease. However, despite this distinguished history, no major advances in the ophthalmic aspects of Graves' disease have been forthcoming. Although a great deal about the thyroidal form of Graves' disease is understood, much about the etiology of the ophthalmic disease is still unknown. However, there have been recent advances which we should all be aware of.

The Thyroid Gland. The thyroid sits in the neck encircling the larynx. It is not there to keep the larynx warm. Rather, it provides thyroid hormone for all the tissues of the body, which may then deiodinate T4 to T3, the active hormone. The thyroid gland is normally under direct pituitary control via thyroid-stimulating hormone (TSH) (Fig. 1). Knowing whether or not the thyroid is under pituitary control is a useful way of thinking about thyroid function testing. The thyroid tissue itself is made up of follicles of specialized epithelial cells that secrete thyroglobulin (Tg) as a colloid into the center of each follicle. The thyroglobulin contains iodinated thyroid hormones (principally T3 and T4), which have been synthesized on the Tg backbone, depending on the availability of iodine and the amount of thyroid hormone needed to be synthesized. The Tg material is reabsorbed through the thyroid cell, the thyroid hormone cleaved off and released as free thyroid hormone and Tg into the circulation. This synthesis and transport of thyroid hormones and Tg is under direct TSH control.

Prevalence of Thyroid Disease. Publications from the Thyroid Foundation of America, a patient advocacy group, stress that thyroid dysfunction is a common abnormality; some 10 million Americans have abnormal thyroid function, and many more have thyroid adenomas (Table 1). In fact, among the population aged over 80 years, a normal thyroid gland is unusual. From epidemiologic studies it appears that Graves' disease has a prevalence of approximately 2%, although there is a 5:1 ratio of females to males and two peaks of incidence in the groups aged 20–40 years and 50–70 years.

Autoimmune Thyroid Disease. There are four autoimmune diseases of the thyroid that we would now group together:

- hyperthyroid Graves' disease (classic Graves' disease with thyrotoxicosis)
- ophthalmic Graves' disease, ophthalmic abnormality with or without thyroid disease
- autoimmune thyroiditis, a term which encompasses two clinical diseases: Hashimoto's disease or goitrous thyroiditis—a goiter must be present; and autoimmune thyroiditis without a goiter, or atrophic thyroiditis, what some physicians still call "primary myxedema"
- the most recently described variant of autoimmune thyroid disease, the syndrome of postpartum thyroid disease (2), usually in the form of atrophic thyroiditis, which may be preceded by thyroid cell necrosis causing high thyroid hormone levels, and which is usually transient. Such patients often, however, develop permanent disease later in life.

Adapted from the author's Grand Rounds in Medicine presentation on March 13, 1990 at the Mount Sinai School of Medicine. From the Division of Endocrinology and Metabolism, Department of Medicine, Mount Sinai School of Medicine, New York, NY. Address reprint requests to the author, Director of The Division of Endocrinology and Metabolism, Department of Medicine, Mount Sinai School of Medicine, Box 1055, One Gustave L. Levy Place, New York, NY 10029.

To help in thinking about the etiology of these diseases, note that Graves' disease and Hashimoto's disease are diametrically opposite in their clinical presentation (Fig. 2). Graves' disease is caused by thyroid-stimulating antibodies and helper T-cells which promote thyroid antibody secretion, resulting in thyroid cell growth and thyroid hyperfunction. On the other hand, thyroiditis is a destructive disease mediated primarily by cytotoxic T-cells and secondary destructive antibodies.

Robert Graves (1796–1853). The description of Graves' disease in 1835 was probably the least significant contribution to medicine that Robert Graves made. A recent biography is entertaining reading (3). Robert Graves was one of the great clinicians of the 19th century. His students went on to teach a wide variety of international medical figures, including Osler, so that the Graves tradition of clinical description lives on today. At the height of his career he was one of the greatest living Irish physicians of the time, and Dublin, where he lived, was a major center of European medicine. He published one of the early, and certainly one of the most useful, clinical textbooks of medicine, which was widely read and translated into a number of European languages during his own lifetime. He, like many physicians at that time, was a general physician but by necessity a cardiologist. He noted a number of patients with heart failure who had goiters and prominent, staring eyes, what John Updike calls "thyroid bulge" (4). An experienced medical writer, he published these cases quickly, in 1835 (5).

Callard Parry. But in 1825 Callard Parry, an English physician with a successful practice in one of the wealthier parts of England, had discovered this same syndrome of heart failure, goiter, and staring eyes and described his cases in his diary. Like many of us, however, he did not bother to publish his cases; in fact his son wrote up the case histories some ten years after the report made by Robert Graves. Since that time a great deal has been learned about the etiology of thyroid Graves' disease, from its treatment by surgery, to the use of iodine by Plummer, and the introduction of antithyroid drugs and radioiodine after World War II (Fig. 3).

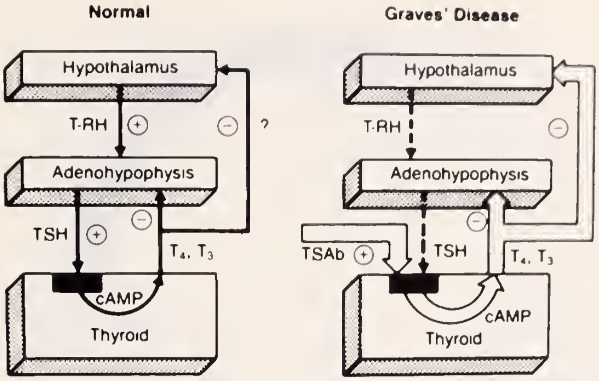


Fig. 1. Pituitary thyroid axis in normal persons (left) and patients with Graves' disease (right). In Graves' disease, thyroid gland is no longer under pituitary control.

Diagnosis. Classically, the disease named for Graves has been defined as clinical and chemical hyperthyroidism associated with a normal or increased 24-hour uptake of iodine 131 in the presence of a diffuse radioactive thyroid scan. Clinically, a family history or the presence of eye disease can often substitute for the nuclear medicine testing, since the gland has a characteristic clinical presentation as smooth, diffusely enlarged, and soft. Histologically, the thyroid tissue shows thyroid epithelial cell enlargement, diminished colloid stores, and diffuse lymphocytic infiltration (Fig. 4).

Today, however, many of us use a different

TABLE 1

Epidemiology of Graves' Disease (per 10,000)

	Prevalence			Incidence		
	Females	Males	All	Females	Males	All
Wickham, UK	190–270	16–23	11–16	20–30	—	—
Olmsted Co, USA	—	—	—	3.7	0.8	2.0
Oakland, USA	54	0	31	7.8	2.1	5.2
Denmark						
Old			9.8			
Young			1.4			
S. Africa						
Black			—			0.4
Colored			—			1.4
White			—			2.3
Japan			8.0			

Adapted from ref. 12.

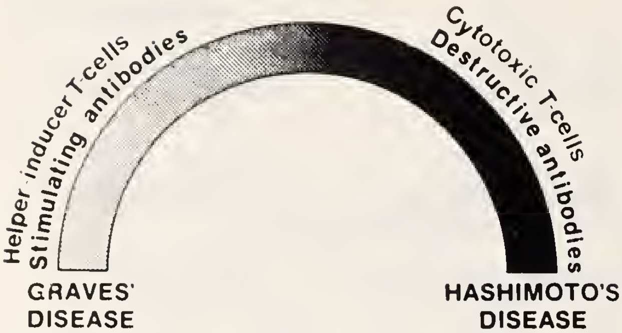

Helper inducer T-cells
Stimulating antibodies
Cytotoxic T-cells
Destructive antibodies
GRAVES' DISEASE
HASHIMOTO'S DISEASE

Fig. 2. Contrasting "rainbow" of Graves' disease and Hashimoto's disease.

definition. The cause of Graves' disease is the presence of stimulating autoantibodies to the TSH receptor (TSHR-Ab). Since the testing for TSHR-Ab is of low cost and widely available, I now define Graves' disease as hyperthyroidism in patients with TSH receptor antibodies in their serum. This approach, unfortunately, leaves a number of patients with the diagnosis of idiopathic or autoimmune hyperthyroidism, depending on the timing and sensitivity of the TSHR-Ab testing. Such TSHR-Ab–negative patients have so far been poorly investigated but clinically do not often fit the classic spectrum.

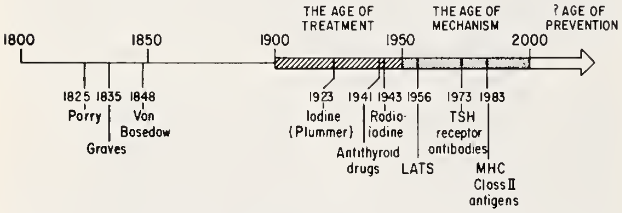

THE AGE OF TREATMENT
THE AGE OF MECHANISM
? AGE OF PREVENTION
1800
1850
1900
1950
2000
1825 Parry
1835 Graves
1848 Von Basedow
1923 Iodine (Plummer)
1941 Antithyroid drugs
1943 Radio-iodine
1956 LATS
1973 TSH receptor antibodies
1983 MHC Class II antigens

Fig. 3. Timeline for Graves' disease. LATS, long-acting thymal stimulator.

Cause of Hyperthyroid Graves' Disease. In 1956 Adams and Purves (6), working in Dunedin, New Zealand, at the University of Otago, discovered thyroid-stimulating antibodies in the sera of patients with Graves' disease and in so doing described the first of the human autoimmune diseases. They used sera from patients with the worst cases of Graves' disease so they could define their assay technique using radioiodine discharge from guinea pig thyroids. Since that time we have come to understand what thyroid-stimulating antibodies are and how unique they are to humans, with no imitations throughout the animal kingdom. Thyroid-stimulating autoantibodies are immunoglobulins of the G class (IgGs) which bind to the TSH receptor on the surface of the thyroid epithelial cells and act as TSH agonists (Fig. 1). Hence the thyroid gland is no longer under pituitary TSH control but becomes stimulated by these autoantibodies, which are known to be secreted within the thyroid gland by the B cells of the thyroidal lymphocytic infiltrate. TSHR-Ab are clonally restricted on the basis of light chain usage and IgG subclass (7, 8), which suggests that they are etiologic in the disease. They are absent in the normal population and found only in patients with autoimmune thyroid disease. TSHR-Ab are detectable in the serum of 90% of patients with hyperthyroid Graves' disease.

Inheritance. It has been known for a long time that Graves' disease and all its corollaries are familial. Patients may have thyroiditis and their familial relations may have hyperthyroidism. Although susceptibility to autoimmune thyroid disease is commonly familial, it remains unpredictable. There are associations with certain HLA allotypes, particularly HLA-DR3 for Graves' disease and HLA-DR5 for autoimmune thyroiditis (9). However, the risk ratios are small, two- to three-fold, so that the presence of HLA-DR3 provides a two- or three-fold increase in the risk of developing Graves' disease. This weak association suggests that the gene for susceptibility to Graves' disease is still some way away from the HLA-DR3 gene. Recent family studies have confirmed these early data and demonstrated an association with HLA antigens but no linkage (10). These types of analyses support the concept of an additional factor that initiates the disease that would account for the association with the HLA gene region which presumably confers susceptibility.

Antigens. The three major autoimmune thyroid antigens have each been cloned and sequenced. These three thyroid-specific antigens

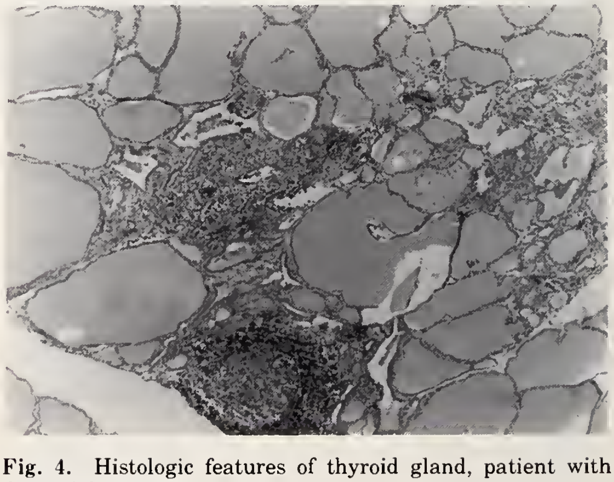


Fig. 4. Histologic features of thyroid gland, patient with Graves' disease.

are thyroglobulin (Tg), thyroid peroxidase (TPO), and the TSH receptor (TSHR). Table 2 summarizes their molecular characteristics. Note that thyroid peroxidase used to be known as "microsomal antigen" and is responsible for the iodination of thyroglobulin.

Sequencing of the thyroid antigens has reintroduced the concept of "specificity crossover" which has been demonstrated in other autoimmune diseases (11). The term "specificity crossover" implies that a monoclonal antibody, for example, may interact with more than one antigen because of the presence of recurrent epitopes in the antigen. Hence, if there are recurrent binding sites on a variety of different tissues, particular antibodies and particular T cells may recognize a variety of tissues. Antibodies to thyroid peroxidase, even murine monoclonal antibodies to thyroid peroxidase, appear to crossreact with leukocyte myeloperoxidase. Antibodies to thyroglobulin, including monoclonal antibodies to thyroglobulin, have been described which crossreact with acetylcholinesterase and all the ramifications that may have for muscle function. Such a recurrent epitope may not be confined to the tissues of the body. For example, antibodies to the TSH receptor appear to crossreact with *Yersinia enterolitica* (for review see reference 12).

TABLE 2
Human Thyroid Antigens and Their Genes

Antigen	Size (Kd)	Amino acids	Site	mRNA (kb)	Chromosome no.
hTSHR	120	744	membrane	4.4 & 4.6	14
hTg	660	2746	secreted	9.0 & 0.9	8
hTPO	107	933	membrane	3.2 & 3.4	2

The major Graves' disease antigen is the human TSH receptor. The TSH receptor appears to conform to all the characteristics of a G-protein-linked membrane receptor (Fig. 5). There are 7 transmembrane elements in the receptor, a significant extracellular domain and a small intracellular tail (13). The G-protein-linked family of receptors includes the adrenergic receptors, the LH receptor and the FSH receptor, which induce the generation of cyclic AMP as their intracellular second messenger. The extracellular portion of TSH receptor is large, which may be one reason it is so antigenic. Clinically, autoantibodies to the LH receptor in humans are uncommon, but autoantibodies to the TSH receptor are present in most patients with Graves' disease.

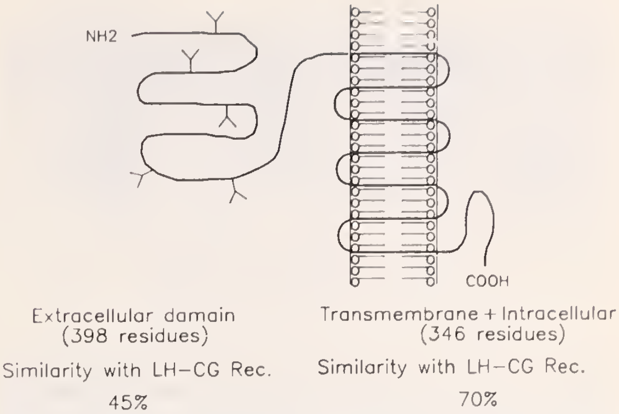

NH2
COOH
Extracellular domain
(398 residues)
Similarity with LH–CG Rec.
45%
Transmembrane + Intracellular
(346 residues)
Similarity with LH–CG Rec.
70%

Fig. 5. TSH receptor structure deduced from cDNA sequence (13).

Measurement of TSHR-Ab. Although there are many different ways to measure TSHR-Ab—thyroidologists love inventing tests of thyroid function—the two currently important ways (Fig. 6) are the TSH radioreceptor assay, which is cheap and reproducible, and the thyroid cell bioassay, which is expensive and has higher interassay variations. Basically, the receptor assay computes either TSH or patient's antibodies with radiolabelled TSH to produce a dose response inhibition effect. In the bioassay, rat thyroid cells or cryopreserved human thyroid cells are stimulated by TSH or serum from the patient, and cyclic AMP, or thyroglobulin release, or another metabolic parameter is measured. These two assays do not measure the same action of TSHR-Ab. The TSHR-Abs in hyperthyroid Graves' disease are thyroid-stimulating antibodies, and they bind well to the receptor and stimulate cyclic AMP accumulation and thyroid hormone secretion. Hence, such antibodies will be detected by either

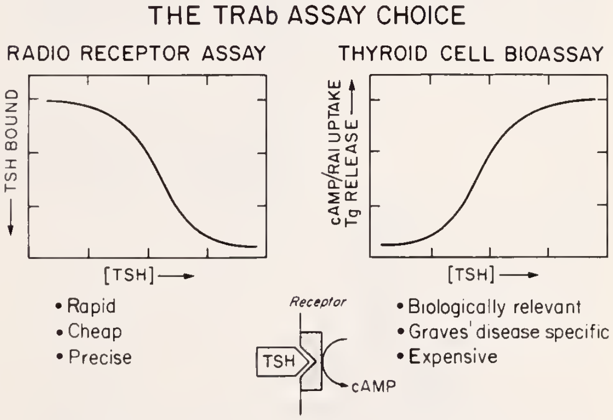


Fig. 6. Contrasting assays for TSHR-Ab.

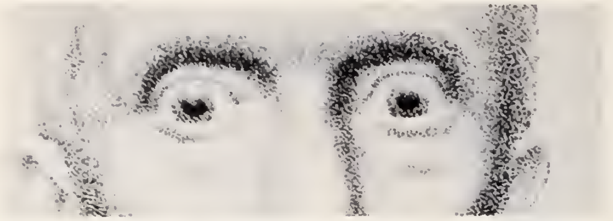


Fig. 7. Example of thyroid stare caused by excess thyroid hormones that is not a sign of Graves' ophthalmopathy.

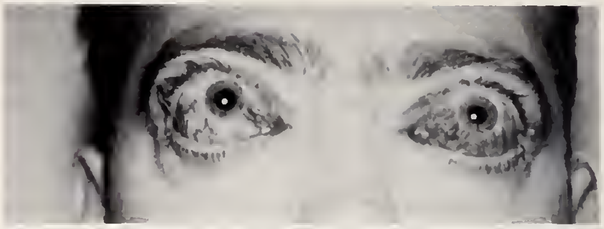


Fig. 10. Signs of ophthalmic Graves' disease.

of these two assay systems. Thus a thyrotoxic patient can serve as the bioassay, and all that is needed is the rapid, cheap, precise radioreceptor system. I have recently summarized the clinical indications for receptor and bioassays in the measurement of TSHR-Ab (14). Essentially we believe the patient is his or her own bioassay. If the patient is hypothyroid, the TSHR-Abs are TSH antagonists or only weak agonists. If the patient is hyperthyroid, the TSHR-Abs must be true TSH agonists. Only in pregnancy is a bioassay occasionally needed if the mother has TSHR-Ab and no functioning thyroid (for example after radioiodine ablation).

Eye Signs of Hyperthyroidism. Thyroid hormone causes thyrotoxic stare, and thyrotoxic stare is induced by thyroid hormone and has nothing at all to do with ophthalmic Graves' disease (Fig. 7). Lid lag and lid retraction, the signs of thyrotoxic stare, occur in guinea pigs given excess thyroid hormone and in any humans given thyroid hormone. They occur in patients with Graves' disease and in patients with toxic multinodular goiter. These signs are not diagnostic of ophthalmic Graves' disease.

Ophthalmic Graves' Disease. The orbit of the eye is a bony cave (Fig. 8). Within the cave, when there is tissue swelling, a variety of mechanical obstructions ensue in addition to disease of the affected tissues. The hallmark of ophthalmic Graves' disease is markedly enlarged orbital muscles (Fig. 9). There is also a marked expansion of retroorbital fat and interstitial tissues. This combination leads to proptosis and vascular compromise with varying degrees of retroorbital and periorbital edema (Fig. 10). There is no muscle destruction, at least not early in the disease, just muscle swelling and muscle dysfunction. Histologically, there is lymphocytic infiltration between often well-preserved, but swollen, muscle cells. Only late in the disease do muscle atrophy and fibrosis ensue as the inflammatory reaction subsides.

The clinical definition of ophthalmic Graves' disease is based on the presence of periorbital edema, conjunctival edema, proptosis, muscle dysfunction, and as the disease progresses, rising intraocular pressure, and optic nerve embarrassment with loss of color vision, peripheral field limitations, and potential blindness. For many years clinical endocrinologists have used subjective parameters to monitor this disease (Table 3). There are many publications on different classifications of the eye changes in Graves' ophthalmopathy, but the most popular classification has been that pioneered by Sidney Werner (15) when he was at Columbia University.

After this crude clinical assessment, the next investigation should be a CAT or an MRI scan to document the muscle bulk. It would also be important to know whether the patient has thyroid disease (using a sensitive TSH assay) and whether the thyroid disease is serum TSHR-Ab positive or negative. Most of all, however, the rate

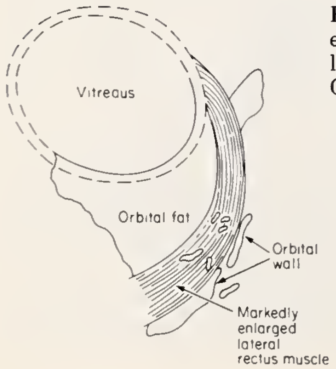
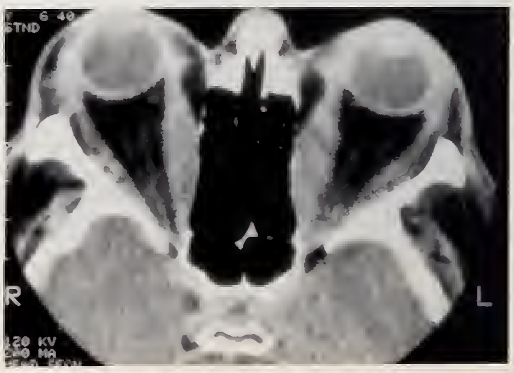


Fig. 8. (Left) Structure of orbit and extraocular muscle. Fig. 9. (Right) Enlarged extraocular muscles seen on CAT scan.



of change in a patient with ophthalmic Graves' disease provides the most useful information.

Graves' Disease Not an Example of Nondeletional Tolerance. Essentially, modern immunologic concepts are designed to explain the emergence of an immunocompetent T cell or a B cell (Fig. 11). What is thought to happen normally is that an immature cell (for example in the thymus) may meet a specific antigen which it is programmed to recognize. If this occurs, it is deleted. If the immature cell does not meet the antigen it is specifically programmed to recognize, it becomes a mature cell. Once the cell is mature and meets its programmed antigen, an immune response may ensue. T cells see their antigen in association with an HLA molecule (16) as a single complex and T cells only interact appropriately with self-HLA. In thyroid disease, for example, the immature T cell may meet Tg processed and presented by an antigen presenting cell (APC) complexed with self-HLA within the thymus. If it is programmed to recognize Tg, it will be deleted. If, however, the immature T cell does not meet Tg, it will become a mature cell. Such mature T cells will be able to react to Tg presented appropriately in the periphery, and induce secretion of Tg-Ab from competent B cells. They may also be anergized by inappropriate presentation of Tg in the absence of the second signals which a competent APC provides to the T cell. In contrast, B cells do not see antigen in the context of HLA. Therefore, if an immature B cell meets Tg, it will also be deleted. If it fails to meet Tg and becomes a mature cell, in the circulation it will inevitably meet Tg and, in the absence of B-cell second signal, will become anergized. If this B cell is, however, also stimulated appropriately by T cells that provide the second signal for B cells, the cell will be able to secrete Tg-Ab rather than entering the state of anergy.

This balance between competent and anergized T cells and B cells is what has been called nondeletional tolerance, as opposed to the simple deletion of immature T cells and B cells in the thymus. We have no need to invoke suppressor T cell defects in this model.

Autoimmune thyroiditis may be a state of nondeletional tolerance, since T cells and B cells reactive to thyroid antigen are present in many normal individuals and animals. In Graves' disease, however, there is a unique autoantibody, and perhaps unique controlling T cells, reactive to TSHR antigen and not found in the "normal" population or even in relatives of patients with Graves' disease. Hence, there is a basic abnormality; what is often called a "forbidden clone" or a clone which is normally kept totally suppressed. The forbidden clone must occur by escaping deletion because it is an unusual cell or perhaps because the deletion system is leaky in particular families based on their HLA endowment, a phenomenon often termed "holes in the immune repertoire." The escaped clone then secretes or directs the secretion of TSHR-Ab that causes thyroid hormone excess, inhibits TSH secretion, and induces a hypermetabolic state.

TABLE 3
Abridged Classification of Eye Changes in Graves' Disease

Class*	Definition†
0	No physical signs or symptoms
1	Only signs, no symptoms (signs limited to upper lid retraction, stare, and lid lag)
2	Soft-tissue involvement (symptoms and signs)
3	Proptosis
4	Extraocular muscle involvement
5	Corneal involvement
6	Sight loss (optic nerve involvement)

* Each class usually, but not necessarily, includes signs and symptoms in preceding class.

† First letters of each definition constitute the mnemonic NO SPECS. Apart from helpfulness for those who use mnemonics for recall, the NO indicates the usually nonthreatening prognosis of classes 0 and 1 (class 1 was formerly termed "mild" or "noninfiltrative" ophthalmopathy), and SPECS, the serious nature of class 2 through 6 (formerly "severe" or "infiltrative" ophthalmopathy).

Adapted from ref. 15.

Causes of Ophthalmic Graves' Disease. Hence, our present understanding is that in ophthalmic Graves' disease a particular abnormal clone, or clones, escape the normal immune mechanisms, and are not deleted. This cell not only recognizes a thyroid antigen but shows "specificity crossover" with retroorbital antigens such as fibroblasts and eye muscle (17). In a series of normal and Graves' disease patients whose serum immunoglobulins have been exposed to a preparation of retroorbital muscle extract (Fig. 12), the patients with severe ophthalmic Graves' disease show antibodies interacting with retroorbital muscle, and such autoantibodies may crossreact with thyroid antigens.

Recently, a 64Kd antigen has been cloned from muscle and thyroid tissue and may represent the ophthalmic antigen hypothesized here. There is also evidence that the retroorbital interstitial fibrous and adipose tissues may be involved, but these data require confirmation. Autoantibodies to an ophthalmic antigen may fix complement and lyse muscle cells in a form of antibody-dependent complement-mediated cytotoxicity. However, the prevalence of the antibod-

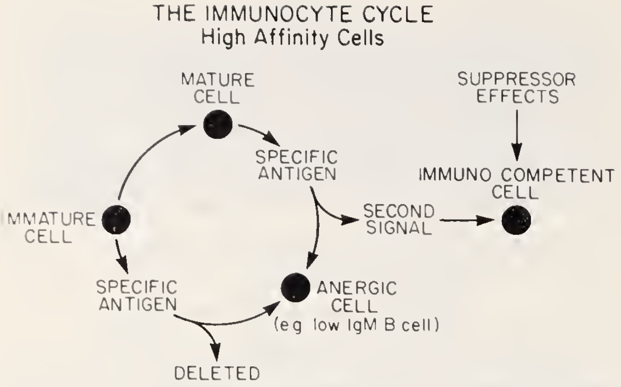


Fig. 11. Plan of anergy and deletion for T cells or B cells (*Reprinted* with permission from ref. 16).

ies in ophthalmic Graves' disease is difficult to know at this time because patients have such varied initial signs and symptoms and because the techniques for measuring antibodies remain imprecise. Such antibodies may also be secondary phenomena rather than forbidden clones, while antigen-specific T cells cause the primary damage. At present, however, little is known of retro-orbital antigen-reactive T cells as initiators of the disease.

Clinical Presentation of Ophthalmic and Thyroidal Graves' Disease. The vast majority of patients have eye disease at the time of being thyrotoxic. However, a significant number have eye disease a long time before or after becoming thyrotoxic, perhaps dependent on the volume of their orbital cavity. The eye disease tends to have its own drumbeat unrelated to thyroidal status. Patients with Graves' ophthalmopathy may, therefore, be divided into various clinical groups:

- The classical Graves-Parry presentation of hyperthyroidism with ophthalmic disease.
- Subclinically hyperthyroid patients with eye involvement and suppressed TSH levels detected with supersensitive TSH immunoassays.
- Patients who have eye disease and normal thyroid function, termed euthyroid ophthalmic Graves' disease. In such patients it is not possible to demonstrate any thyroid abnormality at all.
- Eye disease and hypothyroidism, most commonly following thyroid ablation with radioiodine or surgery. However, the natural history of Graves' disease without thyroidal ablation is still hypothyroidism, as sometimes occurs in those patients who have taken antithyroid drugs for many years (18).

Treating the Hyperthyroidism. The results of a review of some 300 patients observed in long-term follow-up after antithyroid drug treatment, subtotal thyroidectomy or radioiodine treatment (19) are shown in Table 4. The number of patients consulting a physician for the first time with new eye disease following each treatment was in the same range, 5%–7%—but that was only in patients without clinically significant eye disease before treatment. If the patient has eye disease and hyperthyroidism, this easy philosophy does not apply. A number of studies show that patients who have eye disease and hyperthyroidism may deteriorate considerably when treated with either surgery or radioiodine. When patients have thyrotoxicosis and ophthalmic Graves' disease it is, therefore, best to avoid ablating the thyroid with radioiodine and also to avoid thyroid surgery if possible. Treatment for 6 to 12 months with antithyroid drugs allows the eyes to settle, and in the presence of severe disease may be the long term treatment of choice.

How can radioiodine, for example cause such a deterioration? The titer of TSH receptor antibody rises in the 2–3 months after a dose of radioiodine (20). This is thought to be secondary to a combination of thyroid tissue destruction and inhibition of suppressor T cells within the thyroid gland because they are more sensitive to inhibition by the local radiation than helper T cells with a consequent outpouring of antibodies. If the earlier hypothesis that crossreactive autoantibodies and T cells are responsible for ophthalmic Graves' disease is correct, the logical outcome of radioactive iodine therapy should be exacerbation of the eye signs and symptoms.

A recent controlled clinical trial of radioiodine treatment of ophthalmic Graves' disease from Pisa in Italy (21) investigated patients with mild ophthalmopathy rather than severe disease. One group of patients received radioiodine, the other group received radioiodine and concomitant treatment with prednisone in an attempt to prevent this immune response that follows radioiodine therapy. This study (Fig. 13) showed once again that radioiodine worsened the ophthalmopathy indices under controlled conditions, but treatment with corticosteroids at the time of radioiodine treatment and for the following 3 months (20–40 mg/day for one month and then tapered) was helpful in preventing such deterioration. In contrast, many studies have now shown that the antithyroid drugs PTU and methimazole are themselves effective immunosuppressants. They inhibit total IgG secretion, including thyroid autoantibody secretion, at concentrations which are reached within the thyroid when you treat patients with antithyroid drugs (22). Treat-

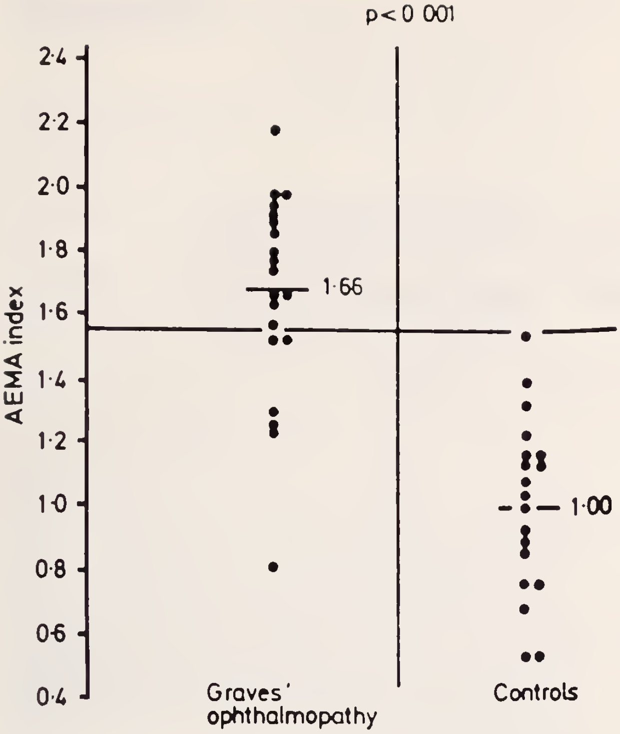


Fig. 12. Assay for antibodies to soluble muscle antigens (from ref. 24). Data expressed in terms of an index measuring amount of antieye muscle antibody (AEMA). The greater the index, the more autoantibody is claimed to be present. Bars, mean indices.

ing patients with antithyroid drugs is, therefore, not only good for lowering the thyroid hormone levels, but also interrupts the immune diathesis. For example, when serum TSHR-Ab is monitored in a variety of different patients, from 50% to 70% of patients will lose their TSH receptor antibodies in response to antithyroid drug treatment over 6 to 12 months.

A General Approach to the Treatment of Graves' Ophthalmopathy. It is usually best to treat the associated thyroid disease first and to provide local measures to reduce ophthalmic inflammation. Working closely with an ophthalmologist who has an interest in thyroid eye disease is always helpful. Patients should be advised to raise the head of their beds to reduce the nocturnal collection of edema, to use artificial tears every 1–2 hours, and to wear dark glasses. Diuretics are usually unhelpful. Time must be allowed for the disease to settle. This is exactly what happens in the majority of patients who are handled conservatively, although it may take over 12 months to occur. We give systemic immunosuppressive therapy only if there is marked deterioration in the eye signs. This may take the form of corticosteroids and/or cyclosporine (23), and a number of additional techniques, such as retroorbital irradiation or exchange transfusions, are in use. Surgical decompression produces excellent cosmetic results in the best surgical hands but should be reserved for quiescent periods of the eye disease. Decompression may be necessary in the active stage when the patient has deteriorating vision but thankfully this situation remains uncommon.

TABLE 4

Incidence of Posttreatment Exophthalmos among Patients Who Did Not Have Clinical Evidence of Infiltrative Ophthalmopathy Pretreatment

Treatment group	No. of cases	% Developing exophthalmos posttreatment
Medical	104	6.7
Surgical	83	7.1
Radioiodine	144	4.9

Adapted from ref. 19.

Summary and Conclusions. Graves' disease is a common, familial, but easily treated condition caused by unique autoantibodies which act as TSH agonists. Patients with the diagnostic eye signs of Graves' disease, however, require more careful treatment than those patients with principally thyroidal involvement because destructive therapy to the thyroid gland may exacerbate the eye disease. The cause of ophthalmic eye disease appears to be "crossover specificity" by the immune system for a recurrent antigen in extraocular muscles, fibroblasts, and the thyroid gland. Treatment directed at the autoimmune response may offer the most appropriate therapy in the years to come.

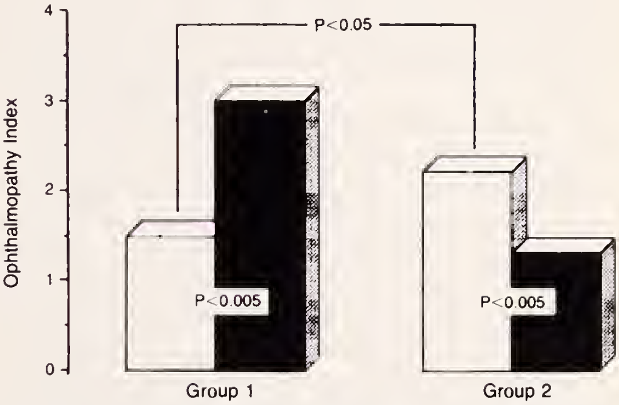


Fig. 13. Corticosteroids are effective in reducing exacerbation of Graves' eye disease after radioiodine therapy. Group 1, no prednisone; group 2, 4 weeks high-dose prednisone as described (21). Group 1 patients showed distinct exacerbation of eye disease after radioiodine therapy, not seen in patients treated with prednisone.

References

1. Quimby EH, Feitelberg S, Silver S. Radioactive isotopes in clinical practice Philadelphia: Lea & Febiger, 1958.
2. Amino N, Miyai K. Postpartum autoimmune endocrine syndromes. In: Davies TF, ed. Autoimmune endocrine disease. New York: Wiley, 1983:247–272.
3. Taylor S. Eponymists in medicine: Robert Graves, the golden years of Irish medicine. London: Royal Society of Medicine, 1990.
4. Updike J. Rabbit is rich. New York: Alfred Knopf, 1982.
5. Graves RJ. Newly observed affection of the thyroid. London Med Surg J 1835; 7:515–523.
6. Adams DD, Purves HD. Abnormal responses in the assay of thyrotropin. Proc Univ Otago Med School 1956; 34: 11–12.
7. Zakarija MJ. Immunochemical characterization of the thyroid-stimulating antibody (TSab) of Graves' disease: evidence for restricted heterogeneity. J Clin Lab Immunol 1983; 10:77–85.
8. Weetman AP, Yateman ME, Ealey PA, et al. Thyroid-stimulating antibody activity between different immunoglobulin G subclasses. J Clin Invest. 1990; 86:723–727.
9. Farid NR, Bear JC. The human major histocompatibility complex and endocrine disease. Endocr Rev 1981; 2:50–86.
10. Roman SH, Greenberg D, Rubinstein P, Wallenstein S, Davies TF. Genetics of autoimmune thyroid disease: lack of evidence for linkage to HLA within families. J Clin Endocrinol Metab 1992; 74:498–503.
11. Abbas AK, Lichtman AH, Pober JS. Cellular and molecular immunology. Philadelphia: W.B. Saunders, 1991.
12. Kendler DL, Davies TF. Immunological mechanisms in Graves' disease. In: Bona C, Siminovitch K, Theofilopoulos AN, Zanetti M, eds. The molecular pathology of autoimmunity. New York: Harwood Academic, 1992 (in press).
13. Parmentier M, Libert F, Maenhaut C, Lefort A, Gerard C, Perret J, Van Sande J, Dumont JE, Vassart G. Molecular cloning of the TSH receptor. Science 1989; 246: 1620–1622.
14. Davies TF. Thyroid-stimulating hormone receptor antibodies—the calm after the storm after the calm? Mayo Clin Proc 1988; 63:736–739.
15. Werner SC. Modification of the classification of the eye changes of Graves' disease. J Clin Endocrinol Metab 1977; 44:203–204.
16. Davies TF. The complex role of epithelial cell MHC class II antigen expression in autoimmune thyroid disease. Autoimmunity 1990; 8:87–89.
17. Wall JR, Salvi M, Bernard NF, Boucher A, Haegert D. Thyroid-associated ophthalmopathy—a model for the association of organ-specific autoimmune disorders. Immunol Today 1991; 12:150–153.
18. Wood LC, Ingbar SH. Hypothyroidism as a late sequela in patients with Graves' disease treated with antithyroid agents. J Clin Invest 1979; 64:1429–1436.
19. Sridama V, DeGroot LJ. Treatment of Graves' disease and the course of ophthalmopathy. Am J Med 1989; 87:70–73.
20. McGregor AM, Petersen MM, Capiferri R, Evered DC, Rees Smith B, Hall R. Effects of radioiodine on thyrotrophin binding inhibiting immunoglobulins in Graves' disease. Clin Endocrinol 1979; 11:437–444.
21. Bartalena L, Marcocci C, Bogazzi F, Panicucci M, Lepri A, Pinchera A. Use of corticosteroids to prevent progression of Graves' ophthalmopathy after radioiodine therapy for hyperthyroidism. N Engl J Med 1989; 321: 1349–1352.
22. Weetman AP, McGregor AM, Hall R. Evidence for an effect of antithyroid drugs on the natural history of Graves' disease. Clin Endocrinol 1984; 21:163–172.
23. Prummel MF, Mourtis MP, Berghout A, Krenning EP, van der Gaag R, Koornneef L, Wiersinga WW. Prednisone and cyclosporine in the treatment of severe Graves' ophthalmopathy. N Engl J Med 1989; 321: 1353–1359.
24. Faryna M, Nauman J, Gardas A. Measurement of autoantibodies against human eye muscle plasma membranes in Graves' ophthalmopathy. Br Med J 1985; 290: 191–193.

Grand Rounds

Established and New Uses of Intravenous Immunoglobulin

Charlotte Cunningham-Rundles, M.D., Ph.D.

Most physicians realize that injections of human immunoglobulin are useful in certain clinical circumstances, yet ten years ago no physician in the United States had used intravenously administered gammaglobulin (IVGG). In the past decade the new IVGG immunoglobulin products have been tried with increasing bursts of enthusiasm. Major hospitals in the United States currently devote millions of dollars to the purchase of these products. Is this expenditure justified? Physicians are using them for a number of diverse diseases, but for which diseases is IVGG now considered an appropriate therapy?

Ten years ago, clinical immunologists approached IVGG in a simplistic way. In clinical immunology we see patients who don't make antibodies. Thus IVGG was a logical therapy; if patients do not have antibodies, let us give them IVGG. About one year into these trials, it developed that clinicians were seeing a number of things which could not be accounted for on the basis of antibody replacement alone. Those are the new uses that I want to touch on.

History of Preparations and Early Uses

Intravenous gamma globulin preparations are produced from human plasma. Human immunoglobulin, or gamma globulin, is a serum concentrate of immunoglobulin fractions that contains IgG1, 2, 3, and 4 with traces of IgM and IgA. Gamma globulin therapy did not start as a treatment for patients who do not make their own antibodies. The first experiments date to more than a hundred years ago, when von Behring and Kitisato (1) found that the antibody fractions of blood were located in the plasma fraction. Further, it was found that immunoglobulin harvested from serum and concentrated antibody preparations conferred the same benefits as preparations harvested from whole blood. Another dramatic advance was the Cohn-Oncley preparation produced in the 1940's (2), by a process that made isolation and concentration of pooled immune globulins possible on a large scale.

In blood fractionation, plasma is harvested and the cryoprecipitate is removed. By using several alcohol fractionation steps, a fraction called Fraction II precipitate, which is concentrated immunoglobulin, is produced. About 95% of Fraction II is IgG, but traces of other immunoglobulins and other plasma proteins are also incorporated. These preparations were found useful for disease prophylaxis, for example in measles and hepatitis.

Early Studies of Uses. In 1952 some patients with immune deficiencies were found to lack immunoglobulin in their blood; immunoglobulin concentrate (Fraction II) was first given as treatment for this condition in that year (3). Subsequently other patients with primary immune deficiency were also treated successfully (4). This treatment, given intramuscularly, is painful and inconvenient and does not raise gamma globulin levels to normal. Some courageous scientists—looking back we can identify the act as courageous because we know what happened—tried to give the intramuscular preparations intravenously. As a result, several deaths occurred, be-

---

Adapted from the author's Grand Rounds in Medicine presentation at Mount Sinai School of Medicine on October 9, 1990. From the Department of Clinical Immunology, Mount Sinai Medical Center, New York. Address reprint requests to the author, Associate Professor of Medicine and Pediatrics, The Mount Sinai Medical Center, Department of Clinical Immunology, Box 1089, One Gustave L. Levy Place, New York, NY 10029.

TABLE 1
Intravenous Immunoglobulin Preparations Licensed in the United States, 1991

Trade name	Manufacturer	Preparation	Product formulation	Comments
1. Gamimune-N	Cutter Laboratories	Acid (pH 4.25) in final product	5% in maltose; liquid form	Replaced original Gamimune.
2. Sandoglobulin	Swiss Red Cross	Acid (pH 4.0) traces of pepsin	3% or 6% contains sucrose, lyophilized	
3. Gammagard	Baxter-Hyland	Ion exchange chromatography	5% contains glycine, lyophilized	IgA and IgG_4 depleted
4. IVEEGAM	Immuno AG	Immobilized trypsin, sequential PEG preparation	5% contains glucose, NaCl, PEG, lyophilized	IgG_3 low IgA reduced
5. Venoglobulin-I	Alpha Therapeutics	PEG fractionation, DEAE-Sephadex ion exchange	5% contains D-mannitol, albumin, PEG; lyophilized	
6. Polygam	American Red Cross	Ion exchange chromatography	5% contains glycine, albumin, glucose, PEG; lyophilized	IgA depleted
7. Gammar IV	Armour Pharmaceutical Company	Ion exchange chromatography	5% contains albumin, sucrose; NaCl; lyophilized	IgA reduced

PEG, polyethylene glycol.

cause these solutions contained aggregates which could activate the complement system, leading to intravascular collapse.

Intravenous Immunoglobulin Products

When immunoglobulin is given subcutaneously, only 33% of the administered dose enters the blood stream by day 7. When it is given intramuscularly, the dose delivered arrives earlier, and the peaks are somewhat higher. But when it is given intravenously, the total dose is delivered to the blood, which then equilibrates with the extracellular fluids; 70% of the dose is present at day 1. Thus intravenous administration of immunoglobulin is the more efficient route.

Much research on methods to produce suitable intravenous immunoglobulins was initiated in 1950–1970 (5–9) which percolated into scientific knowledge two decades later. By using various detergents and solubilization or enzyme procedures to keep immunoglobulins from aggregating, satisfactory intravenous preparations were made by a number of procedures, including different fractionation schemes, additives, and stabilizers.

In 1981 a consensus conference held by the World Health Organization established the standards for the newly emerging IVGG preparations (10). The list of requirements included

- a wide spectrum of antibodies
- a half-life equal to that of normal immunoglobulin
- immunoglobulin in monomeric form
- all immunoglobulin subclasses present
- preparations should not contain any pyrogens or aggregates
- possibly a low IgA level, since some patients have antibodies against IgA
- products should be sterile, stable, and inexpensive.

This is an idealized shopping list that has never been fully achieved.

Considering that one decade ago no intravenous preparations were available in the United States at all, it is remarkable that now there are seven (Table 1).

Uses in Immunodeficiency

The diseases for which replacement immunoglobulin are needed are in fact relatively common, and include hypogammaglobulinemia, antibody deficiency, severe combined immunodeficiency and Wiskott-Aldrich syndrome, X-linked agammaglobulinemia, IgG subclass deficiency, IgA and IgG2 subclass deficiency.

The number of people who have primary immune deficiency disease is said to be approximately the same as the number of people diag-

TABLE 2

Indications for Intravenous Gammaglobulin

Illness	Usual dose
Immunodeficiencies	
Primary antibody deficiencies	200–400 mg/kg/mo
X-linked agammaglobulinemia	more if pulmonary disease or recurrent infection
Common variable immunodeficiency	
IgG subclass deficiency	
Antibody deficiencies	
Combined antibody and cellular deficiencies	
Severe combined immunodeficiency	
Wiskott-Aldrich syndrome	
Secondary antibody deficiencies	
Burns	
Chronic lymphocytic leukemia	
Nephrotic syndrome	
Protein-losing enteropathy	
HIV infections	200–400 mg/kg
Autoimmune disorders	
Acute ITP	400 mg/kg for 5 days; as needed single doses thereafter; may be used with steroid therapy
Chronic ITP	Same as above; repeat courses often needed for up to 6 mo
Neutropenia	Same as above; results last 2–4 weeks
Kawasaki syndrome	400 mg/kg for 5 days
Cytomegalovirus pneumonia* prophylaxis after bone marrow transplantation	Dosages not established

* Several studies indicate IVGG containing CMV antibodies reduces CMV infection and CMV pneumonia during transplant; IVGG not indicated for established CMV infection.

nosed each year with lymphoma and leukemia. Excluding selective IgA deficiency, the number is at least 1 in 10,000 (11). Some of these diseases are very rare; others are more common. Antibody deficiencies can also develop in the course of another clinical illness, either as a part of the disease or because of the treatments used. Table 2 lists the diseases for which antibody deficiency has been demonstrated.

Including all patients who have antibody deficiency for whatever reason, the chances are that every general practitioner is treating someone who has one or more low serum immunoglobulin levels, whether the deficit has or has not been identified.

Dosage. Clinical trials using IVGG in immunodeficiency were begun in 1979. The first question was, how did one decide what the appropriate dose should be? One way was by reference to the doses used in standard intramuscular immunoglobulin treatment. For a person who has no immunoglobulin in their blood, the dose was (and is) 25 mg/kg per week, given biweekly (12). This dose was determined in early trials. Even though the Medical Research Council in England found in an early trial that 50 mg/kg per week produced a better outcome, injections of this size were too large and were clinically unacceptable. Thus the first dose used for IV therapy was 100 mg/kg given per month, a figure picked by prorating the recommended lower intramuscular dose. Subsequent trials in immunodeficiency have used other doses. In our trials in hypogammaglobulinemia we used 300 mg/kg per 3 wks because European experience had showed that this dose was useful (5–6). Higher doses have been tried since this time.

The results in these early trials were mixed. For the lower dose, the results showed that intramuscular immunoglobulin was similar in efficacy to IVGG (13). On the other hand, when 300 mg/kg was given every three weeks, dramatic results were seen (14).

The advantages of intravenous immunoglobulin (IVGG) are obvious (Table 3). Fewer infections are reported; antibiotics are required less often. Patients remark about the diminishment of pain. A physician can achieve any level of serum IgG chosen. There is a lower risk of infection and bleeding. The peak levels are reached rapidly.

IgG Subclass Deficiency. After the above studies were completed, similar studies in IgG subclass deficiencies were performed. When considering these deficiencies, it is useful to recall the different biologic activities of the various IgG subclasses. IgG1 and IgG3 bind complement by either the classic or the alternative pathway. These immunoglobulins cross the placenta. IgG4 does not bind complement well by the classical pathway. IgG subclasses also have different capacities to bind to cells. The IgG2 subclass contains substantially more antibody to bacterial polysaccharides than do the other subclasses. IgG1 and IgG3 are directed primarily against various proteins, viruses, toxins.

From a clinical point of view, patients who have IgG2 deficiency demonstrate the most antibody deficiency and the most frequent bacterial illnesses; in such patients, clinical efficacy of IVGG is much more likely to be demonstrated. The use of IVGG in IgG1 deficiency can also be readily appreciated, since the bulk of IgG is of the IgG1 class. As for deficiency in IgG3 or IgG4, the value of replacement is arguable, since no specific immunologic function has been identified for these proteins.

Lymphoproliferative Disease. In addition to patients who have congenital immunodeficiencies, there are patients who are immunodeficient due to the disease process itself (burns, nephrotic syndrome, hematologic diseases) or to treatment (chemotherapy or immunosuppressives). In chronic lymphocytic leukemia, for example, hypogammaglobulinemia is common in stage 3 and 4 disease. Patients with chronic lymphocytic leukemia of any stage often have little antibody activity to many common bacteria in their blood. In a multiphase trial reported on three years ago (15), it was demonstrated that patients with this form of leukemia had fewer infections when treated with IVGG than patients in the control group who received placebo. Currently there is an ongoing second study in multiple myeloma.

Immune Thrombocytopenia. Shortly after the start of trials of IVGG in immunodeficiency, one of the patients treated in Switzerland was a child who had hypogammaglobulinemia and idiopathic thrombocytopenia purpura (ITP). After treatment, not only did the patient have more antibodies in his blood, as expected, but the platelet count soared. Subsequently, the results of a trial of the use of IVGG in ITP published in 1981 by Paul Imbach (16) showed that in all patients, after an induction dose of IVGG (0.4 g/kg per day for 5 days), the platelet count rose dramatically. While IVGG can often raise the platelet count in ITP, the overall results in ITP show that a few patients (perhaps 10%–20%) will go into remission and never require any further therapy. About 25% will become stable without therapy and about 25% will need intermittent maintenance IVGG. Some patients' conditions are simply refractory (17).

TABLE 3

Advantages and Disadvantages of Intravenous Immunoglobulin as Compared to Intramuscular Preparations

Advantages	Disadvantages
Less painful	More expensive
Fewer injections needed	Venous access necessary
No sterile abscesses	Longer administration time
No mercury exposure	
No limit on dosage size	May result inmyalgias, fever, chills, etc.
Rapid attainment of any desired blood levels	Hepatitis (a few cases) in clinical trials using nonlicensed products
Daily treatment possible	
Can be used in patients who have thrombocytopenia	

How does IVGG work in ITP? ITP is an acquired condition in which there is shortened lifespan of platelets due to autoimmune destruction. There are usually increased numbers of megakaryocytes in the bone marrow. Platelet-associated IgG and anti-platelet antibodies are the hallmarks of the illness. The hypothesis is that for unknown reasons the patient makes an antibody against platelet antigens. These antibodies coat the circulating platelets which are then removed by binding to the monocytes and macrophages of the reticuloendothelial system by adherence of the antibody to the immunoglobulin receptors of cells, Fc receptors. IVGG, however, given in high doses, can produce an Fc receptor blockade, so that platelets coated with immunoglobulin are not removed.

The best demonstration of the fact that IVGG can produce a blockade of the reticuloendothelial system, however fanciful that sounds, is the performance of an experiment in which patients who have ITP and are Rh positive are given a small dose of Rh immunoglobulin-coated red blood cells before and after an infusion of IVGG (18). After IVGG, the clearance of these coated red blood cells is slowed by 200% to 400%. How long does the blockade last? After intravenous immunoglobulin has been given, Fc receptor blockade lasts up to 21 or more days. However, this clearly does not completely explain why IVGG works in ITP, because some patients go into remission, and yet the blockade lasts for only 21 days. The antiplatelet antibodies are not diminished by this process; thus other causes must be considered.

Other Autoimmune Cytopenias. IVGG is certainly of use in autoimmune neutropenia, as we were the first to show (19). In patients who have autoimmune hemolytic anemia, for unexplained reasons reasonable responses often require treatment with extremely high doses, usually for a prolonged period. However, a few reports have been published of good remissions after IVGG treatment in such patients (20).

Mechanism in Immune Modulation. In ITP, blockade of the reticuloendothelial system is definitely present, but, as touched on above, there are probably many other mechanisms of action. Potentially, there are anti-idiotypic antibodies in IVGG which could bind to antiplatelet antibodies, for example, and thus make these antibodies unavailable for binding to platelets.

Some form of immunomodulation best explains the beneficial effects of IVGG in patients who have Kawasaki disease (21), in whom IVGG can apparently prevent coronary artery disease. Kawasaki disease, which is primarily a childhood illness, includes fever, bilateral conjunctivitis, rash, mucus membrane inflammation, erythema of the palms and the soles, and usually lymphadenopathy. No laboratory studies can distinguish this disorder. The standard of therapy has been aspirin until recently, when IVGG was added; in one multicenter trial (20), coronary disease developed in 8% of the IVGG-treated group but in 23% of the control group.

What is the treatment mechanism here? Are there immunomodulating effects, or other mechanisms which are not yet known? It is true that after immunoglobulin infusion, the serum of hypogammaglobulinemic patients can suppress B cells of nondeficient persons from producing immunoglobulin in response to a B-cell activator, pokeweed (22). Changes in T-cell suppression and reduced natural-killer-cell activity have also been demonstrated after IVGG (23, 24). What about the possibility of anti-idiotype interactions? Much work about this seems to show that IVGG contains antibodies which can bind to antibodies directed to the neuromuscular tissues, acetylcholine receptor antibodies, and antimuscle antibodies (25, 26). For these reasons, IVGG has been used in the treatment of myasthenia gravis, Guillaume-Barre syndrome, and polymyositis.

In Infants. Infants in the first three or four months of life are antibody deficient. Premature infants, who do not get the benefit of transplacental passage of antibody, are certainly immunodeficient. Studies have been somewhat conflicting, but a role for the use of IVGG in premature infants has been demonstrated and is still being investigated (27, 28).

Bone Marrow Transplantation. Although few case studies have been done, the burden of evidence shows that IVGG can successfully be used as prophylaxis against cytomegalovirus pneumonia after a bone marrow transplantation (29, 30).

Adverse Effects. About 5% of patients with hypogammaglobulinemia have infusion reactions. In the hypogammaglobulinemic patients, reactions are more common in those patients who have never been previously treated. These reactions include myalgias, fever, shortness of breath, or muscle cramps. Patients who have ITP, hemolytic anemia, autoimmune neutropenia, or Kawasaki disease rarely have infusion reactions similar to those experienced by hypogammaglobulinemic patients, but possibly because of the higher doses, headaches are common side effects. For patients who have antibodies against IgA, a product low in IgA can be successfully used.

Transmission of Virus. There have been cases of non-A, non-B hepatitis after the use of investigational immunoglobulins. The last group of such patients were treated with a preparation made in Scandinavia (31). It is still not certain how the hepatitis C virus could have contaminated this IVGG. No currently licensed products in the United States have ever transmitted hepatitis. HIV infection from IVGG has never been documented. The methods of preparation of IVGG can eliminate HIV by many logs, in fact to the point of noninfectivity (32).

Summary

We know much about the biologic activities of the immunoglobulin molecule. We know its structure, and much about its function. However, I think that surprises are still ahead of us. As we become more familiar with what the immunomodulation doses of IVGG can do, I think we will find other uses of IVGG relying on antiidiotypic antibodies and possibly other cell-mediated functions.

References

1. Von Behring E, Kitasato S. Ueber das zustandekommen der Diptherie Immunitat und der Tetanus-Immunitat bei Thieren. Dtsch Med Wochenschr 1990; 16:1113–1115.
2. Cohn EJ, Strong LE, Hughes WL Jr, et al. Preparation and properties of serum and plasma proteins: 4. A system for the separation into fractions of protein and lipoprotein components of biological tissues and fluids. J Am Chem Soc 1946; 68:459–475.

3. Bruton OC. Agammaglobulinemia. Pediatrics. 1952; 9: 722–728.
4. Janeway CA, Rosen FS. The gammaglobulins: 4. Therapeutic uses of gammaglobulin. N Engl J Med 1966; 275: 826–831.
5. Barandun S, Kistler P, Jeunet F, et al. Intravenous administration of human gammaglobulin. Vox Sang 1962; 7:157–174.
6. Barandun S. Die gammaglobulin-therapie. Chemische, immunologische und klinische Grundlagen. Bibl Haemat 1964; 17:1–138.
7. Schultze HE, Schwick G. Uber neue Moglichkeiten intravenoser Gammaglobulin-Applikation. Dtsch Med Wochenschr 1962; 87:1643–1650.
8. Sgouris JT. The preparation of plasma-treated immune gammaglobulin for intravenous use. Vox Sang 1967; 13:71–84.
9. Stephan W. Beseitigung der Komplementfixierung von gammaglobulin durch chemische Modfizierung mit B-Propiolacton. Z Klin Chem Biochem. 1969; 7:282–286.
10. Appropriate uses of human immunoglobulin in clinical practice: Memorandum of an IUIS/WHO meeting. Bull WHO (Geneva) 1982; 60:45–47.
11. Stiehm ER. Immunologic disease of infants and children. Philadelphia: WB Saunders 1989.
12. Hill LE. Clinical features of hypogammaglobulinemia. Medical Research Council Special Report Series SRS 1971; 310:106–107.
13. Ammann AJ, Ashman RF, Buckley RH, et al. Use of intravenous gamma-globulin in antibody immunodeficiency: results of a multicenter controlled trial. Clin Immunol Immunpathol 1962; 22:60–67.
14. Cunningham-Rundles C, Siegal FP, Smithwick EM, et al. Efficacy of intravenous gammaglobulin in humoral immunodeficiency disease. Ann Intern Med 1984; 101: 435–439.
15. Cooperative Group for the Study of Immunoglobulin in Chronic Lymphocytic Leukemia. Intravenous immunoglobulin for the prevention of infection in chronic lymphocytic leukemia: randomized controlled clinical trial. N Engl J Med 1988; 319:902–907.
16. Imbach P, d'Apusso V, Hift A, et al. High dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. Lancet 1981; 1:1228–1231.
17. Bussel JB, Kimberley RP, Inman RD, et al. Use of intravenous gammaglobulin in patients with chronic ITP. Blood 1983; 62:480–486.
18. Fehr J, Hoffmann V, Kapperler U. Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high-dose intravenous gammaglobulin. N Engl J Med 1982; 306:745–750.
19. Pollack S, Cunningham-Rundles C, Smithwick EM, et al. High dose intravenous gammaglobulin for autoimmune neutropenia. N Engl J Med 1982; 307:253.
20. MacIntyre EA, Linch DC, Macey MG, et al. Successful responses to intravenous gammaglobulin in autoimmune haemolytic anaemia. Br J Haematol 1985; 60: 387–391.
21. Newberger JW, Takahashi M, Burns JC, et al. The treatment of Kawasaki syndrome with intravenous gammaglobulin. N Engl J Med 1986; 315:341–343.
22. Stohl W, Cunningham-Rundles C, Mayer L. *In vitro* introduction of T cell-dependent B cell differentiation in patients with common varied immunodeficiency. Clin Immunol Immunopathol 1988; 49:273–282.
23. Bussel JB, Pahwa S, Porges A, et al. Correlation of in vitro antibody synthesis with the outcome of intravenous gammaglobulin therapy in chronic ITP. J Clin Immunol 1986; 6:50–56.
24. Engelhard D, Wanner JL, Kappor ND, et al. Effect of intravenous immune globulin on natural killer activity: possible association with autoimmune neutropenia and idiopathic thrombocytopenia. J Pediatr 1986; 108:77–81.
25. Roifman CM, Schaffer FM, Wachsmuth SE, Murphy G, Gelfand EW. Reversal of chronic polymyositis following intravenous immune serum globulin therapy. JAMA 1987; 258:513–515.
26. Arsura EL, Bick A, Brunner NG, Namba T, Grob D. High dose intravenous immunoglobulin in the management of myasthenia gravis. Arch Intern Med 1986; 146:1365–1368.
27. Sidiropouios D, Boehme U, Murali GV, et al. Immunoglobulin supplementation in prevention or treatment of neonatal sepsis. Pediatr Infect Dis 1986; 5:185–189.
28. Haque KN, Zaidi MH, Haque SK, et al. Intravenous immunoglobulin for prevention of sepsis in preterm and low birth weight infants. Pediatr Infect Dis 1986; 5: 622–629.
29. Condie RM, O'Reilly RJ. Prevention of cytomegalovirus infection by prophylaxis with an intravenous hyperimmune, native, unmodified cytomegalovirus globulin. Am J Med 1984; 76:134–141.
30. Winston DJ, Ho WG, Lin TH, et al. Intravenous immune globulin for prevention of cytomegalovirus infection and interstitial pneumonia after bone marrow transplantation. Ann Intern Med 1987; 106:12–18.
31. Bjorkander J, Cunningham-Rundles C, Lundin P, et al. Intravenous immunoglobulin prophylaxis in hypogammaglobulinemia causing liver damage in 16 of 77 patients with hypogammaglobulinemia or IgG subclass deficiency. Am J Med 1988; 84:107–111.
32. CDC. Safety of therapeutic immunoglobulin preparations with respect to transmission of human T lymphotrophic virus type III/lymphadenopathy associated virus infection. MMWR 1986; 35:231–232.

The Art and Science of Consultation

The Art of the Gastrointestinal Consultation:

Some Aphorisms

Henry D. Janowitz, M.D.

The Function of the Consultant. As a student 50 years ago I was taught that the function of the consultant was to do a rectal examination and console the family. Fifty years later I teach that the function of the consultant is still to do a rectal examination, but now console the patient.

How can one console the patient who has any one of the many gastrointestinal disorders which go on for a lifetime? How can one answer the patient who wants to know how we can treat disorders when we know that their causes and cures are still unknown? My flippant answer, which I credit to Oliver Wendell Holmes Sr., is this: "The way to live to a ripe old age is to get a nice chronic illness and take good care of it!" My serious answer is: "In the same way that one treats an illness whose cause and therapy is known, tipping the scale in the patient's favor, and increasing the patient's own resistance and immune restorative mechanisms. Even if pneumococcal pneumonia is treated with antibiotics, it is the body's own scavenger system that takes over and destroys the bacteria."

The Consultation

Looking at the Corners. Just as we should look at the corners of the chest film to see if the gastric air bubble is missing in achalasia of the esophagus, so must we look at the corners of the consultation.

Who comes with the patient? Do not assume a man and a woman are married; do not assume that the fathers and mothers of sick youngsters are still married. In trying to find out who's concerned with the patient's health, look for whether the concern is really caring or a chore. Read the "family romance"; it may give you the clue to the mystery.

Why the Consultation? One of the most difficult things to discover is why the consultation is taking place. What prompted it? Who wanted it? Who initiated it—the doctor, the family, the patient, or all three? Only at the end of the entire consultation may it emerge that the death or sickness of a friend or neighbor has triggered the process.

How the patient sees the purpose of the consultation is probably the most important clue to what the patient's expectations are; they are often the hardest to detect. Sometimes it helps to ask at the end of the history taking, "Now that you've had a chance to tell me your story, what is my job?"

Referrals. Don't be too flattered when the patient tells you how great his or her doctor thinks you are. Translate this as follows: "Since *I* have been unable to solve your problem, only a superconsultant can, so I am sending you to a genius."

Beware of the doctor who comes along to the consultation with his patient, because you may never find out what is wrong with the patient. That doctor wants you to agree with her or his program and diagnosis. Keep him out of the examining room. This will give you a chance to talk to the patient alone.

Consultation in a Hospital. Be on time. Better still, be early. This will give you a chance to read the hospital records and notes undisturbed.

From the Division of Gastroenterology, Box 1069, Department of Medicine, Mount Sinai School of Medicine, New York NY 10029-6574. Address reprint requests to the author at that address.

The wise consultant requests a little time alone with the patient, free of the resident physicians, attending physicians, and interns. The wise referring doctor offers to arrange this himself. Only then may you find out why you are consulted.

Setting the Stage. Not all patients will love you, and you need not love all patients. What psychiatrists call "transference" is useful, but remember that every action may call forth an equal and at times opposite reaction—countertransference, the physician's reaction to the patient.

My experience as a patient convinces me that patients can soon and often early on recognize whether your interest and concern for them is authentic and genuine.

If I have become angry with patients, I believe it is because my irrationality responded to their irrationality.

Realistic Expectation. Avoid exploiting the patient's fantasy about the doctor as medical miracle worker and our own of the doctor as savior.

Medicine as well as politics is the art of the possible.

To create an atmosphere of realistic expectations on both sides of the consultant's desk, point out that there is no supreme court in medicine. The patient can always turn to another physician or institution; a further appeal to another court is possible.

Taking the History. The history taking is the heart of the transaction between patient and doctor. In the days when gastroenterologists took their own x-rays, I always thought they could learn more from the patient in the light of the consulting room than in the darkness of the fluoroscopic or dark room. I still feel this is true, even if the light is at the end of a long tube in the age of endoscopy.

Develop your own style of history taking, one you are comfortable with, which means developing your own styles and adapting them to the patients before you.

Make the patient feel comfortable. Make them sit near you—next to your desk if possible, rather than confronting you across the desk. Reduce the obstacles between him or her and you. If the patient brings family or friends, have them present also, up to the capacity of your office space, but give the adolescent a chance to talk to you alone. I draw the line at crawling, crying babies.

Cardinal Symptoms. Sensations are hard to put into words. Encourage the patient to use a metaphor to describe what the pain is like.

Listening to the Patient. Let the patient tell you his or her history in their own way. It will be faster that way. Allow patients to read their notes to you. Avoid leading questions in the beginning, for they can only lead to your own preconceptions.

Remember the dictum that if you listen to the patient long enough he or she will give you the diagnosis. Dig into the history of medications and verify them with the patient's pharmacist. Drugs taken sporadically or intermittently and forgotten may be crucial in the case of pancreatitis.

Take time to review and record the patient's usual daily routine of eating and working. What are his or her usual meals and their menus? The slightest interest in gastrointestinal patients' nutrition will be rewarded by their prompt confidence.

Summarize the history before proceeding to the physical examination. Have you gotten the story straight?

At the end of the formal history taking, I have three questions that have often earned me Brownie points:

- If you were sitting on *my* side of the desk looking yourself straight in the eye, what would you criticize in the way you are taking care of your health?
- If your spouse were here, what would he or she tell me about you? (If the partner is in the waiting room, bring them in.)
- You are not a doctor, but what do you think is the matter with you?

This last question has often evoked dramatic answers that give important clues to the reason for the consultation.

Believing the Patient. Believe the patient, never more so than when he or she has discovered an abdominal mass. Continue to reexamine the patient. The exogastric component of an endogastric sarcoma easily felt by the patient in her tub bath was felt with difficulty by me.

Physical Examination. Examine the sclerae by daylight, by a window if necessary; it is embarrassing to miss jaundice by yellow electric light.

In functional-sounding syndromes, look carefully for all the physical signs of Graves' disease. On the skin, the gross lesions of erythema nodosum and pyoderma gangrenosum present no problems. The early changes of scleroderma of the face and hands may be more subtle. Note the presence of psoriasis of skin and nails associated with inflammatory bowel disease, especially Crohn's disease. Look for the mottled skin of *liv-*

ido reticularis in pancreatitis. Take skin bruises seriously: there are battered adults as well as children.

If you look at the tongue, you will look at the mouth for the aphthous ulcers of Crohn's disease as well as the rare thickening of lips and gums.

Listen to the abdomen before palpating. Most midline noises are meaningless. The left upper quadrant bruit caused by turbulence of the splenic vessels is classic for cancer of the pancreas. Listen to the flank for the bruit of renal vascular anomalies.

Elicit a succussion splash if you can, but be sure that the patient has not eaten recently. A splash in the left upper quadrant is characteristic of gastric outlet obstruction, but other splashes can be heard throughout the abdomen when small-bowel obstruction of any cause is present. Examine the umbilicus for metastatic cancer.

Gastroenterologists are permitted to listen above the diaphragm. Aortic valvular stenosis may be present and points to arteriovenous malformations along the entire gastrointestinal tract in bleeding of unknown origin. Mitral valve prolapse has been associated with some functional bowel syndromes, especially those of colonic inertia.

For right upper quadrant syndromes, simulating biliary tract disease, use the "hooking" manoeuvre to elicit the pain of the slipped 10th and 11th ribs.

Clubbing of the fingertips and curvature of the nails do occur in small bowel disease and in cirrhosis. But always inquire whether there is a family history of clubbing.

Learn to do a careful rectal examination. Go to the rectal clinic if need be. A head lamp leaves both your hands free. Remember that you are more likely to be consulted for rectal pain, fissure, or hemorrhoids early in your career before you get your first case of malabsorption.

Learn the ABCs of rectal hygiene: cotton wipes, warm water, dusting powder if necessary.

To demonstrate rectal prolapse examine the patient in the squatting position.

You are not a proctologist but patients expect you to give them first aid for their hemorrhoidal pain after making the diagnosis. An old-fashioned remedy, witch hazel diluted 1:1 with cold water and applied with Kotex pad for 20 minutes four times daily, helps a lot.

The Initial Summary. At the end of the physical examination write your findings down. Then list your possible diagnoses in the order of probability. Most errors in diagnosis are errors of omission rather than commission. We don't have a wide enough net.

Further, it is important to jot down your impressions and your possible diagnoses at this point because you will never have another opportunity to do this with as fresh a mind as you can now. Then list your plan of investigation, and parallel it by your plans for treatment. Remember there are important diagnostic lessons to be learned from a therapeutic response and furthermore this will enable you to earn the patient's confidence. Recognize the patient not as a puzzle but as someone who is hurting.

Do something for the patient, even at your first interview. It is especially important for patients who are in the hospital. Do something for them every day, even if it is only ordering a hot-water bottle or having a chat. Remember how eagerly the hospital patient awaits your coming.

Leaving the Patient. The approach to the patient is important, but leave-taking is also important. Give him or her the opportunity of asking you one more question. In a hospital setting, if you don't they will come after you or send the nurse after you. The "one more question, doctor," syndrome, I feel, quite characteristically occurs in patients with ulcerative colitis much more frequently than in patients with Crohn's disease, although I haven't done a statistical study on this.

The Patient Leaving You. How will you know you have achieved a positive transference with the patient in the critical interview? You may not. It may require several meetings to get the real feeling. You should be pleased if the patient says "You may be wrong, doctor, but I know what you think," or "I know that you will forget about me when I leave, but while I'm here you are thinking only about me."

Recordkeeping. Always take the patient's weight. Patients' recollections of weight loss and gain are frequently misleading.

Remembering patients' faces and names, unless you have good visual memory, is difficult, especially when they call on the phone. Having a Polaroid picture of the patient on the front page of the chart has helped me immensely in talking to them on the telephone. The picture is taken at the end of the initial consultation; when its purpose is explained, few refuse permission.

Evaluating Records. Since the consultant's opinion is to be a truly independent one, all agree consultants should take their own history and do their own physical examination of the patient.

What are they to do with the massive records, films, scans, and sonograms that nowadays accompany the patient? They must review the x-rays themselves, and look at and study the ultrasound studies and CT scans with their radiological colleagues, unless they are well versed in these modalities. I expect the consultant to be able to interpret an endoscopic retrograde cannulation of the pancreatic duct (ERCP), a transhepatic angiogram, an intravenous cholangiogram, a colloid liver scan, and the isotopically labelled hepatobiliary scan. Angiography requires expert consultation. Pathologic slides must clearly be reviewed with the appropriate colleagues if they are central to the problem.

More difficult is the question of what to do with the reports of endoscopic examination by others; must one repeat these studies? If the question is critical to decision making, the answer must be yes.

The more common and more anxiety-provoking problem has to do with repeating the gastrointestinal radiographs. Patients are reluctant to be exposed to further radiation, especially if the films have been taken recently. If the problem is a gastroduodenal one, repeating endoscopic examination rather than the radiographs may be a suitable compromise. A negative barium enema can be confirmed by colonoscopy.

The major errors I have committed consisted in not repeating the small bowel films which the patient brought to the consultation or in accepting a gastrointestinal series that did not clearly present the entire small bowel. This is especially important with barium enemas that show no reflux into the terminal ileum.

Symptoms, Signs and Syndromes

Pain. There are a few periodic pains of gastrointestinal origin which awaken patients regularly from sleep. Peptic ulcers of the stomach or duodenum, and cancer of the pancreas, are the commonest causes of pain at night.

The irritable bowel rarely awakens the patient at night.

Proctalgia fugax, a lightning-like, intense rectal spasm, usually occurs in the middle of the night. I classify this pain in the same group as nocturnal leg pains.

The timing of the acute pain of biliary colic or pancreatitis is related to the time of eating the offending meal or drinking the alcohol. For this reason, biliary colic rarely occurs early in the morning.

The classic location of a pain is classic because it so frequently occurs in the expected location, but the atypical location must also be borne in mind. Sigmoid diverticulitis can occur in the right lower quadrant as the pain of acute appendicitis.

Nausea. Nausea, especially nausea without vomiting, is one of the most difficult symptoms to manage and treat. Its mildest form may also be excessive salivation. Organic nausea arises most frequently from the duodenum and is transmitted via vagal afferents; remember that of the 40,000 vagal fibers, 36 or 38 thousand are believed to be afferents to the brainstem.

Although giardiasis causes nausea and the organism does live in the duodenum, it is usually accompanied by small bowel symptoms.

One can't help wondering whether pacemaker problems will not be discovered in the duodenum. "Sick duodenum" may become an established diagnosis. I have sometimes prescribed Dilantin in desperation, but only occasionally with success.

Diarrhea. Getting definitions straight is important in this context. My rough and ready one, "too often and too loose," works most of the time. What to call it when the patient complains of having frequent formed stools in rapid succession?

The separation of osmotic from secretory diarrhea is made quite easily; stopping all oral food intake, replacing it with intravenous fluids, will soon reveal whether the diarrhea is a secretory one. The simpler approach in a patient who is not having bowel movements at night may not always be helpful; patients with the irritable bowel syndrome also have some night stools on occasion.

It is remarkable how often intractable diarrhea stops directly after the patient is hospitalized. He or she is usually the patient with the irritable bowel syndrome.

Secretory diarrhea due to circulating secretagogs of a hormonal nature (VIP, catecholamines, etc.) does exist but is rare. Suspect laxative abuse and surreptitious use of diuretics first. Add some sodium hydroxide to the stool to detect the presence of phenolpthalin.

Gas. My heart sinks when the chief complaint is gas. I fall back on this schematic triad: (a) swallowed air, (b) CO_2 released in the intestine by the interaction of gastric hydrogen ions and bicarbonate in the duodenum from the pancreas, and (c) the noxious gases released by bacterial action on colon food residues, mainly carbohydrates.

Gas analysis of rectal flatus is not needed to separate the former from the latter. The nose will do: odorless versus odiferous.

Bloating and Abdominal Distention. One interesting presentation of a bloating syndrome is the woman who insists she looks as if she were nine months pregnant every afternoon and evening, no matter what she eats or doesn't eat. This is not pseudocyesis; rather I call it pseudo-pseudocyesis due to the extreme arching of the spine as the patient lies on the examining table. Put your hand under her back to demonstrate this. I have found this functional disorder almost impossible to treat.

Constipation. Difficulty in moving one's bowels has become an interesting symptom. Sorting out the effects of chronic laxative use, separating the effect of psychotropic drugs, sorting out the variety of neurogenic and myogenic pseudo-obstructive syndromes has become an intriguing clinical exercise. Incomplete forms of Hirschsprung's disease should be borne in mind; my oldest patient was 61 years.

Anal manometry and intestinal transit marker studies with radio-opaque pellets have helped tremendously to this sorting out. I am not certain I have learned much from defecograms except in rectal prolapse.

Swallowing. Disturbances of swallowing are a tricky area in any patient and especially in the older one. The burden is on us to be sure that the patient does not have a cancer in either the esophagus or the fundus of the stomach invading the esophagus. If a biopsy taken on endoscopy is negative, repeat the biopsy.

Remember that reflux esophagitis can also give symptoms of cough which is not often appreciated. Another sign of reflux of acid content into the esophagus and spilling over into the larynx and lungs is the presence of ulcerations of the arytenoid cartilage of the larynx. You may need a nose and throat consultant to help you find this.

For patients who have difficulty in swallowing, don't forget to inquire for the presence of Raynaud's phenomenon. Patients are not apt to offer this spontaneously and often I need to remind myself to ask the appropriate questions.

All globus is not hystericus and all globus hystericus is not hysteria.

Ask questions such as, Is any food found on the pillow in the morning? I have seen this phenomenon more frequently in patients who have a Zenker's diverticulum than in those who have achalasia, but it can occur in both. The patient who recalled seeing an apple seed on his pillow had a diverticulum.

Review the films that the patient brings to see whether the cervical esophagus is present on the upper gastrointestinal series. I have mentioned the treacherous cancer of the fundus invading the esophagus which is not seen on normal esophagoscopy, and may not be present on biopsy, which I suggest you repeat. However, in these cases a CAT scan of the area has often been helpful.

There are neurological problems of swallowing, especially in the older patient who may or may not have had a stroke, but don't assume that the difficulties of swallowing are a central nervous system defect; these patients can also have cancer.

Dyspepsia. In addition to x-ray negative dyspepsia, we certainly have the syndromes of endoscopic-negative dyspepsia. Bear in mind the fact that the pathophysiology of an ulcer can be present without the histopathology of an ulcer.

The problem of gastric emptying obviously suggests itself to all of us in patients with nonulcer dyspepsia. Bear in mind that barium leaves the stomach quite differently than food does. Barium has a specific gravity greater than one, and is one of the few things that leaves the stomach by gravity. Here the gastric emptying scan of radiolabelled meals with good controls in the same laboratory can be helpful.

Remember that the small lesser curvature gastric ulcer can lead to marked impairment of emptying. Tachygastria, the rare syndrome of alterations in electrical activity of the pacemaker, affecting spontaneous activity of the antrum, has been verified in some patients and can lead to the syndrome of periodic vomiting. Prostaglandin inhibitors such as indomethacin have been used to treat some of these patients. Occasionally, in situations of desperation, I have used Dilantin as therapy; one such case was a notorious success, unfortunately limited by the hypertrophy of the gums that later developed.

Gastric Lesions and Problems. Particularly vexing are those patients who on x-ray have thickened folds of the stomach. Are these thickened folds simply loose flabby folds or are they infiltrated? The usual endoscopic superficial biopsies don't help. Bear in mind that the folds may occasionally represent gastric varices in the absence of esophageal varices. Gastric varices may be present with normal liver function—Orthodox Jews can have cryptogenic cirrhosis, and assimilated Jews can have alcoholic cirrhosis too.

Problems after operation on the stomach are more difficult to manage than those following operation on any other part of the gastrointestinal tract.

Diarrhea after gastric vagotomy is common. Even more common is diarrhea after vagotomy plus cholecystectomy. Bile salt catharsis is my hypothesis. Bile binders often help.

Biliary Tract Disease. All has not been solved by modern endoscopy. In episodes of right upper quadrant pain that don't fit the classic biliary colic syndrome where radiographic studies are negative, perform the "hooking maneuver" to see if there is any pain you can reproduce as a result of disturbances in the 10th and 11th and possibly the 12th rib. Don't assume that the pain is due to the slipped rib just because the hooking maneuver (right or left) elicits pain. Sometimes it is important to have a skillful anesthetist or surgeon do a local block of that area or that rib to demonstrate that the pain does arise from the slipped rib before dissecting out the rib. But if the case has been well documented the results of operation are dramatic.

In patients with clinical signs of classic biliary tract disease, how far do we go to make the diagnosis? Certainly sonography and oral cholecystogram and blood samples drawn at the time of the episode or during the middle of the night at the time of acute episodes are in order. Certainly the first episode would not lead me to do an ERCP, though repeated episodes would, especially if in association with pancreatitis. Remember also there is a place for intravenous cholangiography with tomography. Bear in mind that at times duodenal drainage to search for cholesterol crystals may give one the answer. This has more than an academic interest because it would then be reasonable to treat the patient with ursodeoxycholic acid. It may be necessary to perform this test more than once.

Patients with repeated episodes of classic biliary colic, despite our inability to demonstrate by laboratory studies the presence of stones, may sometimes need to be explored. This may be the last diagnostic method and may be the first therapeutic maneuver.

Bilocular gall bladders are usually quite innocent, but one can in time develop stones in one of the locules. Gallbladders with Ashoff-Rokitansky sinuses may also have or lead to chronic inflammation and stones.

Gallstones or cholesterolosis may develop rapidly in patients with negative laboratory studies, especially following a period of rapid weight loss. I postulate here that mobilization of cholesterol from the tissues during the period of weight loss leads to this.

Pancreatic Disease. The basic problem is to try to decide whether the pain the patient is talking about has anything to do with the pancreas. Usually most clinicians rely on some elevations in serum amylase; the basic question to ask here is whether this amylase is of pancreatic origin. Now that commercial laboratories are doing pancreatic isoamylase studies, this question can be readily resolved. I think this should be looked into quite early on.

Remember some patients with cystic fibrosis can have acute and chronic pancreatitis; they may also have calcification of the pancreas, and this may occur in the older age groups.

In considering the questions of acute recurrent pancreatitis, remember that "common causes" are common: Alcohol use may need some digging out, and biliary tract disease may remain hidden for a while. We all know enough to look for disturbances in lipid and calcium metabolism, but the question of drug-induced pancreatitis is one of the hardest to elicit. The classic example is the doctor's wife who is taking a diuretic for premenstrual tension or to lose weight, but who doesn't take it all the time and who fails to correlate it, since it isn't always associated with pain. Remember that hypertriglyceridemia can occur and be aggravated by the older type of contraceptive pills and may lead to severe pancreatitis.

When one is really desperate one has to look for other causes of acute pancreatitis, such as ductular anomalies. Pancreas divisum seems to have had its day as an explanation of obscure pancreatitis.

The central clinical place for the secretin test is to determine whether the patient has chronic pancreatitis: a persistent lowering of the juice bicarbonate, below 90 mEg/L, is good evidence, and will occur long before there is evidence of diabetes, calcification, or steatorrhea. Unfortunately, few laboratories still do this test.

Cancer of the Pancreas. I have never felt a cancer of the pancreas on abdominal examination; what one may feel is the liver pushed forward. When I have felt a mass in the pancreas, it has been either a pseudocyst or a lymphoma.

Right Lower Quadrant Pain. Sometimes a slow leak from a duodenal ulcer may track to the right lower quadrant down the right gutter and may mimic appendicitis. Recurrent appendicitis secondary to fecaliths does occur. If the x-ray appearance of Crohn's disease of the ileum disappears completely, ascribe the changes to acute appendicitis and/or appendiceal abscess, or yersinial infection. Use the sonogram more frequently; it can be very helpful.

Obscure Gastrointestinal Bleeding. We are all aware of angiodysplasia of the intestinal tract that may be associated with aortic valvular disease, mainly aortic stenosis or idiopathic hypertrophic aortic stenosis. Bleeding has also been reported in conjunction with coarctation of the aorta.

In the small bowel, in patients whose x-rays of the intestinal tract are normal and where there is no blood in the gastric aspirate or on endoscopy nor from the colon as shown by colonoscopy, the commonest missed lesions that I have seen are myomas of the jejunum; sarcoma of the ileum was detected on CAT scan; Crohn's disease of the small bowel not seen on x-ray but in which case a bleeding scan was helpful. The small-bowel enteroscope has been a notable advance of the past few years.

Scanning has not been helpful in bleeding of ulcerative colitis, in contrast to Crohn's disease. However, the labeled red cell scan has been a notable advance, and I would not do an angiogram unless the labeled red-cell scan were positive. Vascular anomalies of the small bowel can bleed and then can stop bleeding and remain silent for years; one just has to keep repeating the red cell scan to find it, or better still the enteroscope.

Some Notes on Inflammatory Bowel Disease. Isolated Crohn's disease involving the duodenum alone is extremely rare. I have made the mistake of calling a duodenal ulcer Crohn's disease. Much more common is the error in which one calls ulceration of the duodenum (in the presence of steroid-treated, proven Crohn's disease of the small or large bowel) a duodenal ulcer. The most likely cause is Crohn's disease, not peptic ulceration.

In looking for the presence of an enteric fistula to bladder or vagina you have to ask specifically whether the patient passes gas in urination or in the tub. In my experience, in Crohn's disease a vaginal fistula occurs almost invariably in the presence of an absent uterus. Almost all these patients have had a hysterectomy, which somehow allows the ileal-vaginal fistulae to develop.

Ulcerative proctitis is much more stubborn than reported in textbooks and often fails to respond to sulfasalazine by mouth, 5 aminosalicylic acid (5-ASA) enemas, and local steroids. These stubborn cases rarely respond to oral steroids and one sometimes has to resort to intravenous ACTH or hydrocortisone. I can envisage the situation in which one might have to use immunosuppressive agents.

Isolated lesions of the small bowel and proximal ileum are rarely Crohn's disease in the absence of terminal ileal disease. Other causes to bear in mind are endometriosis, carcinoid tumor, lymphoma, retractile mesenteritis, and even infarction.

There is such a thing as "chronic toxic dilation of the colon." Patients recover some tone but are slow in developing complete return to normal tone, which may take more time than the usual toxic dilation and may go on for several weeks. If the patient's clinical condition is improving, one should persist in conservative therapy.

Remember that there may be rectal sparing in ulcerative colitis, sometimes as part of the natural history of the disease but most frequently as a result of local rectal steroid or 5-ASA instillations.

I have never seen an enterocutaneous fistula heal and stay healed under medical therapy, including hyperalimentation.

Bladder fistulae do not represent an emergency. Ascending kidney infections do not take place. Massive bleeding, however, may become alarming.

Distinguish between recurrent and residual Crohn's disease after an operation.

Intestinal Obstruction in Crohn's Disease. Intestinal obstruction in Crohn's disease may involve three components: an inflammatory mass, spastic motor phenomena, and fixed stricture. It may take some therapeutic maneuvering to sort these components out. Antibiotics may not help. Do not assume that the response to steroids is proof that inflammatory changes are primary.

Rare Causes of Abdominal Pain and of Intestinal Bleeding. Meckle's diverticulum, most often found in children, may occur in adults; my oldest patient was 54 years old.

Another unusual cause of abdominal pain is retroperitoneal fibrosis. In one patient it was ascribed to the use of reserpine, a well-known serotonin releaser.

The syndrome of retractile mesenteritis shades off into retroperitoneal fibrosis and can at times have a rather acute phase; it can be difficult to separate from pancreatitis with fat necrosis or subcutaneous panniculitis.

Remember also that you don't have to be Jewish to have familial Mediterranean fever.

Postoperative Notes. Although postoperative fever can occur from a variety of extra-abdominal causes, the commonest cause of persistent fever is in the belly, if the belly has been invaded.

In this context, liver abscess is one of the hardest to discover and the slowest postoperative complication to be picked up; sonography and CAT scanning are needed to find it.

Factitious Disease. One always begins by trusting the patient and having faith in his or her good will, just as the patient has faith in our good will. However, we have to think of factitious disease, especially when the pattern of the disease fits no obvious clinical patterns and when one is dealing with a paramedical professional, including nurses and technicians, those who work in hospitals, doctors, and doctors' spouses.

Factitious diarrhea always comes into the problem when one is dealing with what seems to be secretory diarrhea. While tumors secreting hormones (VIP, catecholamines, and some of the other newer peptide fragments which resemble VIP) do exist, they must be extremely rare; much more common is the factitious diarrhea due to surreptitious use of laxatives but also to diuretics. It is difficult to prove; we often have to send the patient off the floor and search the room thoroughly, and even then it may be difficult, perhaps illegal. Obviously, adding some sodium hydroxide to the stool and seeing the red color will indicate the presence of a laxative containing phenopthaline.

I have been deceived by a patient with known Crohn's disease who inflicted skin lesions on herself. Some of the factitious diseases can mimic gastrointestinal disease, such as self-inflicted bleeding from the rectum or from a stoma. Beware of challenging the patient directly unless you are prepared to handle the patient's deep-seated psychological problems.

Infarction of the Bowel. Despite the good circulation in rectal vessels, infarction can take place there, especially if any hypercoaguability of the blood or hyperviscosity is present. I have seen one such case in a physician who had polycythemia.

Intestinal obstruction can occur in the absence of an abdominal scar due to an internal hernia. The paraduodenal and parajejunal ones can certainly be recognized not only on the flat plate but by barium by mouth and the characteristic collection of barium in the right upper and left upper quadrants. A succussion splash can be elicited anywhere over the abdomen in intestinal obstruction.

Other Abdominal Syndromes. The frustrating right lower quadrant pain in a young woman is a well-known syndrome. GI series and small bowel x-ray and barium enema, flat plate of the abdomen, CAT scan and sonography, often fail to reveal any pathology. The patient then has an intensive gynecological examination, including pelvic examination and laparoscopy, to be sure we are not dealing with endometriosis. Some of these patients may have appendicitis. There is an occasional patient in this category who improves following appendectomy performed through the laparoscope although I am not advocating this as a general rule. One of the most baffling of such right lower quadrant pain syndromes, which led the patient to dig at her abdomen while asleep in the middle of the night, was due to a migrating fibroid on a long pedicle.

The Irritable Bowel Syndrome. We have all seen the irritable bowel. We don't know what it is, yet we treat it and worry if we have missed some organic disease. I don't think we should treat this diagnosis as a wastebasket. It is a definite entity. In my conceptual framework, it is a dysrhythmia of the colon. I think lactose intolerance can mimic it, but I don't think lactose intolerance is the whole story. I agree that the Western diet is too refined and may be involved in the pathogenesis of diverticulosis and some forms of constipation. I don't think it is the answer to the irritable bowel.

While physical examination, digital rectal examination, stool guaiac study, barium enema, radiographic gastrointestinal series, small bowel examination, and so forth are all important, it is the history that is most important. What is the irritant in the specific patient's irritable bowel? This means a carefully detailed history of the patient's eating habits, and attention to their lifestyle. Everyone has their own little cookbook remedy.

As far as treatment of the irritable bowel syndrome goes, I think the high fiber diet has marked limitations. Since many patients really cannot take bran, I think we must listen to them. There is an occasional patient who has wheat intolerance, yet doesn't have sprue. The thing that plagues us all is whether there is such a thing as food allergies. Yes, there is, especially in patients who have a strong family history of atopic allergies and an elevated IgE, and it is damned difficult to figure out what specific food items are giving them their problems. One can eliminate lactose and one can eliminate gluten but neither really helps. In desperation, for about 20 years I have been using a "core" diet approach, recently also advocated by English colleagues. For two weeks or more I have the patient drink only bottled mineral water, eat rice or potato, lamb chops or chicken and canned Bartlett pears. I allow them to add one new food at a time, if they are stabilized after two weeks. I abandon this approach if the patient does not significantly improve after three weeks.

Apropos of the crossover between food allergies and malabsorption due to gluten enteropathy or sprue, I think that out of every 10 patients with sprue diagnosed by small-bowel biopsy with the appropriate malabsorptive studies, 7 or 8 will respond beautifully to a gluten-free diet. One patient may require removal of lactose from the diet, another the addition of steroids. Furthermore, some patients with gluten enteropathy do have pancreatic insufficiency and may require the addition of pancreatic enzymes. An occasional patient cannot tolerate eggs or chicken.

In symptomatic therapy of the irritable bowel syndrome, I think there is a place for the smooth muscle blockers and we certainly ought now to try the calcium channel blockers. In some more desperate cases, I advise various forms of relaxation therapy, including counseling and instruction in biofeedback.

Constipation in its extreme form is now an interesting disease. The varieties of “colon inertia,” as I prefer to call it, are extending. Adult or incomplete forms of Hirschsprung’s disease do exist. Anal manometry would certainly help make the diagnosis, but sometimes it is necessary to do a barium enema without preparation to demonstrate the narrowed segment. We are all aware of the variety of neurogenic and myogenic forms of pseudo-obstruction which usually involve the colon and may also involve the small bowel and the esophagus. In the study of patients with profound colonic inertia who don’t fit into the other categories, in addition to manometry to rule out Hirschsprung’s disease, the radiopaque marker study can help us decide if the patient is one who would respond to a colonic resection.

Important in this context is to dig out, while eliciting the history, whether the patient is taking one of the wide variety of psychotropic drugs which cause or worsen constipation.

Secretory Diarrhea. Most cases of secretory diarrhea are factitious, and a surprising number of these patients do feel better as soon as they are admitted to the hospital. If there is any question about secretory diarrhea, I stop all feeding by mouth, place the patient on intravenous therapy of fluid replacement, and collect the stools. This will certainly help us to decide if the patient is having secretory diarrhea. Just asking about night-time diarrhea is not enough. In this context there are some patients who have idiopathic bile salt catharsis. Many of them are older patients, and for some reason or another, possibly due to the alterations in intestinal transport or changes in ileal reabsorption of bile salts, they are pouring larger amounts of bile salts into the colon than that organ can handle, inducing a catharsis. They respond nicely to bile salt binders.

Absorption of Fat-Soluble Vitamins. It is a curious but well-established fact that the absorption of the fat-soluble vitamins A, D, E, and K is disturbed differently in different forms of steatorrhea.

Nightblindness, osteomalacia, and bleeding secondary to hypoprothrombinemia occur in the patient with the fat malabsorption of small bowel mucosal disease (gluten enteropathy, Crohn’s ileojejunitis), but not in the fat malabsorption of chronic pancreatic disease. Micellar formation does not seem to be the limiting factor.

Functional Colonic Obstruction. Remember that there are valves on the left side of the colon other than the anal sphincter. Occasionally I have seen a functional obstruction of the colon mimicking organic obstruction with complete cutoff of rectally instilled barium. Observe the post-evacuation films and the patient for a short while before exploring. These valves will open up spontaneously.

Epilogue

Remember that classical radiography, endoscopy of the stomach and colon, CAT scanning, sonography, and ERCP have not and will not put gastroenterologists out of business; neither will nuclear magnetic resonance or positive emission tomography.

Gastroenterology is still an active transaction between doctor and patient. Gastroenterologists are physicians who know how to think about and manage patients who have gastrointestinal problems.

Suggested Reading

1. Janowitz HD. Your gut feelings. New York: Oxford University Press, 1989.
2. Janowitz HD. Inflammatory bowel disease: a personal view. New York: Field and Rich, 1985, distr. by Year Book Medical Publishing.

Clinical and Genetic Associations in Marfan Syndrome and Related Disorders

FRANCESCO RAMIREZ, PH.D., BRENDAN LEE, PH.D., AND EMILIA VITALE, PH.D.

Abstract

Although Marfan syndrome has been a recognized clinical entity for nearly a century, only in the early 1950s were its manifestations and genetic features firmly and fully established. Similarly, although the identification of the basic defect in Marfan syndrome had been vigorously pursued for several decades, it was only in 1991 that two independent reports implicated fibrillin as the defective gene product. Surprisingly and serendipitously, these studies also revealed genetic heterogeneity of the fibrillin proteins and established linkage between one of these loci and a Marfan-related disorder, congenital contractural arachnodactyly. Like Marfan syndrome, this condition is accompanied by skeletal abnormalities; however, flexion joint contractures replace the loose-jointedness of Marfan syndrome and, more importantly, neither the eye nor the aorta are affected. More recently, a similar association with the fibrillin gene has also been established in the dominantly inherited form of ectopia lentis. These associations of structurally related gene products with Marfan and related syndromes may conceivably imply that other connective tissue disorders are caused by mutations in these or other yet undiscovered fibrillin genes.

Historical Perspective. A French pediatrician, Antoine Bernard-Jean Marfan (1858–1942), described in 1896 a 5½-year-old girl, Gabrielle P., who had disproportionately long limbs and fingers, fibrous contractures of fingers and knees, and tall stature (1). Marfan gave the name "dolichostenomelia" to this clinical presentation to emphasize the unusually long and thin extremities of the young girl (1). Six years later, Gabrielle was reevaluated by H. Méry and L. Babonneix, who added scoliosis to the skeletal abnormalities previously described by A. Marfan (2). In that report, having in mind the contrasting phenotype of achondroplasia, they renamed the condition "hyperchondroplasia" (2). In the same year, another French physician, C. Achard, coined the term "arachnodactyly" (long, spidery fingers) to describe the clinical phenotype of a seemingly identical patient (3). Subsequent reports added congenital displacement of the ocular lens (ectopia lentis) and mitral valve regurgitation to the skeletal features of the disorder (4, 5). The involvement of the connective tissue, together with appreciation of the heritable nature of the syndrome, led H. Weve, in 1931, to propose the name of "dystrophia mesodermalis congenita, typus Marfanis" (6). Seven years later, E. Apert, a student of A. Marfan, shortened Weve's definition to the term in current use, Marfan syndrome (MFS) (7).

It was after Marfan's death in 1942 that the aortic abnormalities were correlated to MFS morbidity and mortality (8, 9). As a result of an extensive study on a large number of MFS patients and their families, in 1955, Victor McKusick published a seminal paper in which he delineated the natural history of this syndrome along with its range of pleiotropism and variability (10). In this study he also firmly established the autosomal dominant mode of inheritance of MFS. Since then, MFS has been recognized as part of a col-

---

From the Brookdale Center for Molecular Biology, Mount Sinai School of Medicine. (Article 97 from the Brookdale Center.) BL is now at Dept. of Microbiology and Immunology, State University of New York, Health Science Center at Brooklyn; EV, at Dept. of Psychiatry, Columbia University. Address reprint requests to Francesco Ramirez, Ph.D., at Box 1126, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029.

lection of connective tissue disorders whose careful clinical dissection has in turn led to an international nosology of this group of heritable conditions (11).

Clinical Manifestations

The current clinical definition of MFS relies on three "major" manifestations: aortic dilatation, ectopia lentis, and typical habitus (12–15). Often, these features are associated with "other" manifestations that affect to varying degrees these and other organ systems, such as the skin and the central nervous system (12–15).

The major manifestations in the skeletal system, composing the so-called Marfanoid habitus, include tall stature with increased arm span and dolichostenomelia of the legs. Other classic manifestations include arachnodactyly and anterior chest deformities (pectus excavatum or carinatum). A long narrow skull (dolichocephaly), malar hypoplasia, scoliosis, dural ectasia, and joint mobility abnormalities are variably manifested in MFS (12–15).

Ocular lesions in MFS are most commonly localized in the lens in the form of congenital dislocations which are almost always bilateral. Iridodonesis (tremor of the iris) is a good indicator of lens dislocation which, in the great majority of cases, is in the upward direction. Severe myopia, retinal detachment, and, rarely, glaucoma are consequences of ectopia lentis and represent the major causes of visual impairment in MFS patients (12–15).

Manifestations in the cardiovascular system are associated with increased morbidity and mortality, life expectancy being reduced to two-thirds that of control populations. Cardiovascular lesions in the form of aortic dilatation, dissection, regurgitation, and congestive heart failure are primarily responsible for over 90% of deaths in patients who have MFS. Early cardiovascular changes often appear initially as mitral valve prolapse and mitral valve regurgitation. Conduction abnormalities (supraventricular and ventricular arrhythmias) are usually detected on EKG examination (12–15).

Pneumothorax, hernias, dural ectasia, characteristic stretch marks in the skin (striae atrophicae), and even neuropsychological problems have recently been associated with the triad of cardinal manifestations of MFS (15). The spectrum of pleiotropism is, however, likely to increase further with additional clinical sequelae. This is mostly because of the increased survival rate of MFS patients resulting from improved therapy and management of the cardiovascular complications.

Genetics and Diagnosis

MFS is currently recognized as a dominantly inherited autosomal disorder of high penetrance. It occurs at an incidence of approximately 1 in 10,000, 25%–30% of the cases representing new mutations. The syndrome displays wide clinical variability both within and between families (15–19). The variability within families presumably reflects the diversity of the genetic background in which the mutations are expressed; variability between families is likely to be the result of allelic mutations. Accordingly, involvement of at least two systems and one major manifestation fulfills the diagnostic requirements in familial cases. In the absence of other affected family members, diagnosis is based on the involvement of the skeleton, and of at least two other systems and one major manifestation (11).

From this and other compendiums, it is obvious that MFS does not exist as an isolated entity, but as a common disorder in a continuous clinical spectrum of overlapping phenotypes. Among others, these phenotypes include homocystinuria, congenital contractural arachnodactyly (CCA), dominantly inherited mitral valve prolapse syndrome, familial annuloaortic ectasia (Erdheim disease), dominantly inherited ectopia lentis (EL), and Stickler syndrome (12, 15). Incidentally and not surprisingly, it is now widely believed that the original patient described by A. Marfan did not fulfill the current criteria for MFS. Indeed, and as first suggested by Beals and Hecht (16), Gabrielle probably suffered from CCA. This rare connective-tissue disorder shares the typical habitus of MFS with the distinguishing feature of joint contractures, rather than loose-jointedness; the ophthalmic and cardiovascular manifestations of MFS are absent (12).

The Search for the MFS Gene. With the recognition of MFS as a heritable disorder of connective tissue, intensive research efforts immediately focused on the extracellular components of the connective tissue, including both fibrillar and nonfibrillar components. Fibrillar components range in diameter from less than 10 nm to greater than 100 nm and include collagenous and noncollagenous macromolecules. Nonfibrillar components, such as the family of proteoglycans and elastin, compose most of the remaining proteins.

The first of these studies focused on the fibrillar collagens, the most abundant structural component of the extracellular matrix (ECM). Reports of increased urinary excretion of hydroxyproline, increased dermal collagen solubility, and decreased borohydride-resistant collagen cross-links originally supported this hypothesis (17–20). Subsequently, however, linkage analyses using collagen gene-specific markers convincingly excluded these proteins from the etiology of MFS (21). Concurrent studies focused on proteoglycan metabolism, defects in hyaluronic acid, β-glucoronidase, and decorin synthesis (22–25). These, like the collagen studies, did not lead to the establishment of causal association with MFS.

In the past few years, a series of electron-microscope analyses have identified a novel ECM aggregate, the so-called 10 nm microfibrils (26). These microfibrils are widely distributed in connective tissue matrices, including the suspensory ligaments of the lens, the periosteum, and the large vessel walls. They are believed to serve at least three distinct functions (26). In elastic tissues, they provide both the scaffolding on which elastin is deposited and the structural protein system that links elastin to other ECM structures. In nonelastic tissues, they appear to have a structural function of their own. The complete inventory of the microfibril constituents is presently unknown. It is also unclear how microfibrils are assembled in the ECM of various organ systems.

Utilizing polyclonal and monoclonal antibodies raised against tissue rich in connective tissue microfibrils, a 350 kD, acidic glycoprotein was identified in 1986 by Sakai et al. (27). The authors named this novel ECM component "fibrillin" and showed that it co-localizes with both elastic and nonelastic microfibrils (27). Compositional analyses revealed that fibrillin contains a high percentage of acidic amino acids and cysteine residues (28). Immediately after secretion into the ECM, fibrillin is believed to promptly become insoluble by a process which is likely to involve the formation of intra- and, presumably, inter-chain disulfide bonds (28).

In the early 1980s, the late David Hollister first suggested that, given its tissue distribution, fibrillin might be the candidate protein in MFS pathogenesis. To test this hypothesis, he performed a series of elegant immunofluorescence studies with fibrillin-specific monoclonal antibodies on the microfibrillar array of nonaffected and MFS individuals. Analyses on sporadic patients and ten MFS kindreds found a consistent decrease in fibrillin content in both skin and cultured fibroblasts of MFS patients (29, 30). These studies, published in 1990, provided the first strong evidence of an abnormal ECM aggregate in MFS. However, this work could not conclusively associate defective fibrillin with the MFS phenotype, since the method of analysis did not discriminate between primary and secondary defects.

Concurrent with these studies, linkage analyses with randomly chosen and gene-specific polymorphic probes were carried out in a large collection of MFS families. This approach was facilitated by the highly penetrant, dominant inheritance pattern exhibited by many large multiplex MFS families. Exclusion of linkage between MFS and various fibrillar and nonfibrillar collagen genes, as well as the elastin gene, were first reported (21, 31–35). These results reiterated that any protein abnormality found in these matrix gene products is a secondary effect of an as-yet-unknown primary defect.

Subsequently, linkage analyses with random probes resulted in an exclusion map of nearly 75% of the human genome for possible location of the MFS gene locus (36). In the same year, 1990, Kainulainen et al. reported the successful localization of the MFS locus to chromosome 15q15-q23 using chromosome-specific, random probes in a genetic linkage study of five Finnish kindreds (37). Shortly thereafter, studies in different ethnic groups confirmed this localization, offered specific physical orders for the anonymous probes in this region, and suggested locus homogeneity within MFS (38–41). Although highly informative, this experimental approach could not, however, elucidate the nature of the gene(s) at the MFS locus and thus independently support the immunohistochemical evidence implicating fibrillin in MFS etiology.

In 1991, a meeting was sponsored by the National Marfan Foundation at the Banbury Center in Cold Spring Harbor to openly discuss the most recent results in the search for the MFS gene. A few months later, data presented at this meeting resulted in three publications from two different research teams confirming the original prediction of D. Hollister that the fibrillin gene and the MFS locus on chromosome 15 are one and the same (42–44). Furthermore, in that body of work evidence was also provided for the existence of additional fibrillin genes (42). One of these genes was shown to reside on chromosome 5 and to be linked to CCA, the disorder affecting A. Marfan's original patient (42). More recently, linkage has been also established between the fibrillin gene on chromosome 15 and another rare MFS-related condition, dominantly inherited EL (our unpublished data).

Fibrillin Proteins. Although still preliminary, the information is already sufficient to allow speculation about the structure and evolution of the fibrillins, as well as about their pathological contribution to MFS and related phenotypes.

The conceptual translation of the cDNAs encoding the fibrillins of chromosome 5 (Fib5) and 15 (Fib15) shows that the two proteins are related in evolution and structure (42). Using the partial sequence of Fib15 as a paradigm, several general conclusions regarding the structure of these glycoproteins can be drawn. On superficial examination, the primary structure of fibrillin can be generalized as being composed of several repeated cysteine-rich motifs (43). These peptide modules can, in turn, be segregated into three distinct groups based on their composition and sequence homology.

Accordingly, the great majority of fibrillin is made of repeats resembling the epidermal growth factor (EGF) motif and with an average composition corresponding to the sequence $X_4CX_6CX_4$-$CX_8CXCX_{12}CX$ (Type I motif) (45). Based on NMR studies of other EGF-containing proteins, such a motif is expected to fold into a structure with a characteristic amino-terminal triple-stranded β-sheet (45). The six cysteines stabilize this structure by forming disulfide bonds in the order: 1 to 3, 2 to 4, and 5 to 6 (45). Interestingly, all of the fibrillin Type I motifs belong to the EGF-CB (calcium-binding) subclass of repeats because of the conserved spacing between, and identity of, four residues within each repeat (45). As a consequence of folding, these amino acids come into close proximity to one another. In other EGF-containing proteins, such an alignment signals the hydroxylation of one of these conserved residues, as well as the formation of a high-affinity calcium-binding consensus sequence (45).

The second most common motif of fibrillin (Type II motif) is analogous to the 8-cysteine module seen in the TGF-β-bp (binding-protein) (46). Like the fibrillin sequence, the TGF-β-bp motif is present only a few times, interpersed among the numerous EGF-like repeats. The consensus sequence of the Type II motif is $X_7CX_8CX_{12}CCCX_g$-$CX_2CX_{11}CX_{17}$, the 3-cysteine cluster being a likely candidate for providing moieties used for the tertiary structural organization of the molecule and/or for interchain bonding.

Similar functions could be envisioned for the least represented fibrillin motif (Type III motif) (42). This novel peptide module consists of a unique 8-cysteine-containing sequence whose composition is $X_6CX_8CX_{12}CCX_{10}CX_2CX_5CX_{11}CX$. Additional structural features of the fibrillin protein are the presence of the cell-binding sequence Arg-Gly-Asp in one of the Type II motifs, and of several potential glycosylation sites along the entire molecule (43).

Fibrillin Mutations. Identification of fibrillin mutations in MFS patients can in theory infer structural-functional relationships of the protein based on the genotypic-phenotypic correlations of the mutations. The first important and still anecdotal observation from these ongoing studies is the absence of deletions in the Fib15 genes of MFS patients (44). This could be explained either by assuming that gross genomic rearrangements occur in the region of the gene so far uncloned, or by postulating that they are associated with either end of the clinical spectrum of MFS that is not yet defined.

The latter hypothesis would imply that point mutations are the most-recurrent events responsible for what is currently recognized as being the MFS phenotype. This hypothesis is indirectly supported by similar evidence from the *Drosophila Notch* locus (47). This gene encodes a polypeptide containing EGF-like repeat which is involved in neurological development. All of the *Notch* mutations analyzed to date are single amino acid substitutions (47). Moreover, analysis of *Notch* variants indicate that seemingly identical EGF motifs are not identical in function, because each mutation results in a quite distinct phenotype (47). Incidentally, half of the EGF repeats of the *Notch* gene are of the EGF-CB subclass (47).

Published and unpublished data of Fib15 mutations in a limited number of MFS cases have until now identified defects leading to three theoretical types of metabolic consequences. The first is a Pro for Arg substitution in a Type I repeat preceding the fourth cysteinyl residue and following the last amino acid of the putative calcium-binding sequence (44). Based on the structural considerations discussed above, one could hypothesize that such a mutation changes the local secondary structure, thus indirectly affecting cysteine-mediated disulfide bonding within both the molecule and the supramolecular aggregate. Alternatively, the change could lead to a local disruption of the calcium-binding site, provided that some or all of the fibrillin repeats have such a function and that the absence of calcium binding is of pathogenic significance in MFS.

The second group of mutations include cysteine substitutions. Conceivably these missense mutations disrupt local folding of the module, or tertiary and quaternary interactions of the protein, or both.

The third and final group of mutations are nonsense mutations. Because the details of fibrillin biosynthesis are essentially unknown, this type of mutation could give rise to two distinct scenarios. In one, a truncated protein alters microfibril assembly by being incorporated into the system. In the other, the truncated protein is excluded from subsequent metabolic steps and the phenotype is caused by the less (50%) than normal biosynthesis of fibrillin. This latter scenario would imply that not only qualitative mutations but also quantitive defects in fibrillin can contribute to MFS pathogenesis.

Fibrillin Genes. Although only small portions of the fibrillin genes have been characterized, it is increasingly evident that the genomic structure replicates the modular arrangements of the proteins (42). Preliminary data indicates that, regardless of its nature, each of the repeats is encoded by a single exon (42). The distribution of the coding sequence in each of these exons is also characteristic, for they all begin with a 2/3 split codon and end with a 1/3 split codon (42). As a consequence of such an arrangement, the overall organization of the gene is that of an in-frame, "multicassette" type.

An interesting corollary to the predicted organization of the fibrillin gene is that deletions and splicing (exon-skipping) mutations will similarly result in the production of transcripts translating normal amounts of shortened polypeptides. These products will be likely to ultimately interfere with the extracellular assembly of the microfibrillar meshwork.

A similar situation, with respect to gene organization and evolution, exists for the family of the fibrillar collagen genes (48). Based on this paradigm, one would predict that the fibrillin gene family followed a similar evolutionary path. Accordingly, the structure of the fibrillin gene may have evolved by duplication of an ancestral exon coding for a Type I motif. After this structure was established, the entire gene duplicated and transposed on different chromosomes.

Present and Future Perspectives

The search for the molecular etiology of MFS has resulted in the discovery of a small family of genes related in evolution, structure, and function (42). The fibrillin genes code for polypeptides of unique structure which individually or collectively form supramolecular aggregates seen as 10 nm microfibrils in the connective tissue matrix (42, 43). These microfibrils are present in diverse tissues, including the ciliary zonule of the eye, the aorta, periosteum, and perichondrium (27). It is still unknown whether the different fibrillin polypeptides interact as subunits within the same supramolecular aggregate and/or exhibit individual, different tissue distributions (26, 27). Finally, it has been documented that mutations in one of the fibrillin genes, Fib15, cause MFS (42, 44). Concurrently, linkage analysis has suggested that mutations within Fib5 and Fib15 may result in two MFS-related phenotypes, CCA and EL respectively (42, and our unpublished data).

Differentiation between the MFS and CCA phenotypes may be explained by the differential roles played by the two fibrillin proteins in affected tissues. To reconcile the contrasting phenotypes seen in the Fib15-linked conditions, analogy may be drawn with type I procollagen mutations in osteogenesis imperfecta and Ehlers-Danlos syndrome type VII (48). These clinically distinct conditions are in fact the result of mutations affecting different structural-functional domains of the same polypeptide (48). A more complete understanding of the metabolic and molecular consequences of fibrillin mutations will, however, necessitate genotype-phenotype correlations derived from the description of a collection of naturally occurring variants. This should also be compared and contrasted with cell biological and biochemical analyses of fibrillin secretion, assembly, and interactions in the ECM.

If additional fibrillin loci exist, as some preliminary evidence suggests (42), it would be of great importance to assess their possible association with other MFS-related disorders. For example, it would be of interest to ascertain this possibility in the familial forms of mitral valve prolapse syndrome and annuloaortic ectasia (49). The delineation of naturally occurring mutations and subsequent derivation of genotype-phenotype correlations will help to evaluate the prognostic value of mutational analyses, as well as to lend insight into the metabolic significance of defective fibrillins. Furthermore, the development of specific antisera will allow for the study of fibrillin tissue distribution, secretion, assembly, and interaction within connective tissue matrix in normal and diseased states. Ultimately, study of fibrillin metabolism and associated disease phenotypes will benefit from the development of transgenic animal models. Finally, the demonstrated molecular homogeneity of MFS paves the way for the use of gene-specific markers in prenatal and presymptomatic diagnosis of members at risk in informative MFS families.

In conclusion, the use of molecular genetic analyses has aided in the delineation of the biochemical defect in a common disorder of connective tissue. It has also uncovered a novel group of genes and suggested an association to a clinical spectrum of systemic disorders. It is safe to predict that deeper future understanding of these clinical-genetic associations will shed new light on the pathophysiology of several organ systems.

Acknowledgements

This review is dedicated to the lasting memory of our friend, David W. Hollister (1941–1991), who gave us the means and determination to complete his work.

We are most indebted to the Elster family for having motivated and generously supported our research effort. We wish also to acknowledge the support of our colleagues at the Brookdale Center for Molecular Biology, particularly that of Ms. R. Lingeza.

References

1. Marfan AB. Un cas de déformation congénitale des quatre membres plus prononcée aux extrémités charactérisée par l'allongement des os avec un certain degré d' amincissement. Bull Mém Soc Med Hop Paris, 1896; 13:220–226.
2. Méry H, Babonneix L. Un cas de déformation congénitale des quatre membres: hyperchondroplasie. Bull Mém Soc Méd Hop, Paris, 1902; 19:671–675.
3. Achard C. Arachnodactylie. Bull Mém Soc Méd Hop Paris, 1902; 19:834–804.
4. Boerger F. Uber zwei Fälle von Arachnodaktylie. Z Kinderheilkd 1914; 12:161–184.
5. Piper RK, Irvine-Jones E. Arachnodactylia and its association with congenital heart disease: report of a case and review of the literature. Am J Dis Child 1926; 31: 832–839.
6. Weve H. Veber Arachnodaktylie (Dystrophia mesodermalis congenita, typus Marfanis). Arch Augenheilk 1931; 104:1–11.
7. Apert E. Les formes frustes du syndrome dolichosténomélique de Marfan. Nourisson 1938; 26:1–12.
8. Etter LE, Glover LP. Arachnodactyly complicated by dislocated lens and death from rupture of dissecting aneurysm of aorta. JAMA 1943; 123:88–89.
9. Baer RW, Taussig HB, Oppenheimer EH. Congenital aneurysmal dilatation of aorta associated with arachnodactyly. Bull Johns Hopkins Hosp 1943; 72:309–331.
10. McKusick VA. The cardiovascular aspects of Marfan's syndrome: a heritable disorder of connective tissue. Circulation 1955; 11:321–342.
11. Beighton P, De Paepe A, Danks D, et al. International nosology of heritable disorders of connective tissue. Berlin 1986. Am J Med Genet 1988; 29:581–594.
12. McKusick VA. Heritable disorders of connective tissue, 4th ed. St. Louis: CV Mosby, 1972, 61–223.
13. Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. N Engl J Med 1979; 300:772–777.
14. Pyeritz RE, McKusick VA. Basic defects in the Marfan syndrome. N Engl J Med 1981; 305:1011–1012.
15. Pyeritz RE. Marfan syndrome. In: Emery AEH, Rimoin DL, eds. Principles and practice of medical genetics, 2nd ed. New York, NY: Churchill-Livingstone, 1990, 1047–1063.
16. Beals RK, Hecht F. Congenital contractural arachnodactyly: a heritable disorder of connective tissue. J Bone Joint Surg 1971; 53-A:987–993.
17. Laitinen O, Uitto J, Iivanainen M, Hannuksela M, Kivirikko KI. Collagen metabolism of the skin in Marfan's syndrome. Clin Chim Acta 1968; 21:321–326.
18. Prockop D. The intracellular biosynthesis of collagen: some possible implications for diseases of bone and other connective tissues. Arch Intern Med 1969; 124: 563–570.
19. Priest RE. Collagen produced by cells cultured from patients with the Marfan syndrome is more soluble than normal. J Cell Biol 1972; 55:208A.
20. Bocek RJ, Noble NL, Gunja-Smith Z, Butler WT. The Marfan syndrome: a deficiency in chemically stable collagen cross-links. N Engl J Med 1981; 305:988–991.
21. Boileau C, Jondeau G, Bonaiti C, et al. Linkage analysis of five fibrillar collagen loci in a large French Marfan syndrome family. J Med Genet 1990; 27:78–81.
22. Appel A, Horwitz AL, Dorfman A. Cell-free synthesis of hyaluronic acid in Marfan syndrome. J Biol Chem 1979; 254:12199–12203.
23. Lamberg SI. Stimulatory effect of exogenous hyaluronic acid distinguishes cultured fibroblasts of Marfan's disease from controls. J Invest Dermatol 1978; 71:391–395.
24. Nakashima Y. Reduced activity of serum β-glucuronidase in Marfan syndrome. Angiol 1986; 37:576–580.
25. Pulkkinen L, Kainulainen K, Krusius T, Mäkinen P, Schollin J, Gustavsson K-H, Peltonen L. Deficient expression of the gene coding for decorin in a lethal form of Marfan syndrome. J Biol Chem 1990; 265:17780–17785.
26. Cleary EG, Gibson MA. Elastin-associated microfibrils and microfibrillar proteins. Int Rev Connect Tissue Res 1983; 10:97–209.
27. Sakai LY, Keene DR, Engvall E. Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol 1986; 103:2499–2509.
28. Maddox BK, Sakai LY, Keene DR, Glanville RW. Connective tissue microfibrils: isolation and characterization of three large pepsin-resistant domains of fibrillin. J Biol Chem 1989; 264:21381–21385.
29. Godfrey M, Menashe V, Weleber RG, et al. Cosegregation of elastin-associated microfibrillar abnormalities with the Marfan phenotype in families. Am J Hum Genet 1990; 46:652–660.
30. Hollister DW, Godfrey M, Sakai LY, Pyeritz RE. Immunohistologic abnormalities of the elastin-associated microfibrillar fiber system in Marfan syndrome. N Engl J Med 1990; 323:152–159.
31. Tsipouras P, Borresen AL, Bamforth S, Harper PS, Berg K. Marfan Syndrome: exclusion of genetic linkage to the COL1A2 gene. Clin Genet 1986; 30:428–432.
32. Dalgleish R, Hawkins JR, Keston M. Exclusion of the α2(I) and α1(III) collagen genes as the mutant loci in a Marfan syndrome family. J Med Genet 1987; 24:148–151.
33. Ogilvie DJ, Wordsworth BP, Priestley LM, Dalgleish R, Schmidtke J, Zoll B, Sykes B. Segregation of all four major fibrillar collagen genes in the Marfan syndrome. Am J Hum Genet 1987; 41:1071–1082.
34. Francomano CA, Streeten EA, Meyers DA, Pyeritz RE. Exclusion of fibrillar procollagens as causes of Marfan syndrome. Am J Med Genet 1988; 29:457–462.
35. Huttunen K, Kaitila I, Savolainen A, Palotie A, Peltonen L. The linkage analysis with RFLP markers of elastin

and type III collagen genes in Finnish Marfan families. Am J Med Genet 1989; 30:244A.

36. Blanton SH, Sarfarazi M, Eiberg H, et al. An exclusion map of Marfan syndrome. J Med Genet 1990; 27:73–77.
37. Kainulainen K, Pulkkinen L, Savolainen A, Kaitila I, Peltonen L. Location on chromosome 15 of the gene defect causing Marfan syndrome. N Engl J Med 1990; 323:935–939.
38. Dietz HC, Pyeritz RE, Hall BD, et al. The Marfan syndrome locus: confirmation of assignment to chromosome 15 and identification of tightly linked markers at 15q15-q21.3. Genomics 1991; 9:355–361.
39. Tsipouras P, Sarfarazi M, Devi A, Weiffenbach B, Boxer M. Marfan syndrome is closely linked to a marker on chromosome 15q1.5 → q2.1. Proc Natl Acad Sci USA 1991; 88:4486–4488.
40. Sarfarazi M, Tsipouras P, Boxer M, O'Connell MP, Child AH, Murphy W. Fine mapping of the region surrounding the Marfan syndrome locus on 15q1.5 → 2.1. J Med Genet 1992; 29:75–80.
41. Kainulainen K, Steinmann B, Collins F, et al. Marfan syndrome: no evidence for heterogeneity in different populations and more precise mapping of the gene. Am J Hum Genet 1991; 49:662–667.
42. Lee B, Godfrey M, Vitale E, et al. Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature 1991; 353:330–334.
43. Maslen CL, Corson GM, Maddox BK, Glanville RW, Sakai LY. Partial sequencing of a candidate gene for the Marfan syndrome. Nature 1991; 353:334–337.
44. Dietz HC, Cutting GR, Pyeritz RE, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 1991; 353:337–339.
45. Davis CG. The many faces of epidermal growth factor repeats. New Biologist 1990; 2:410–419.
46. Kanzaki T, Olofsson A, Moren A, et al. TGF-β1 binding protein: a component of the large latent complex of TGF-β1 with multiple repeat sequences. Cell 1990; 61: 1051–1061.
47. Rebay I, Fleming RJ, Fehon RG, Cherbas L, Cherbas P, Artavanis-Tsakonas S. Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. Cell 1991; 67:687–699.
48. Lee B, D'Alessio M, Ramirez F. Modifications in the organization and expression of collagen genes associated with skeletal disorders. Crit Rev Euk Gene Expr 1991; 1:173–187.
49. Glesby MJ, Pyeritz RE. Association of mitral valve prolapse and systemic abnormalities of connective tissue: a phenotypic continuum. JAMA 1989; 262:523–528.

Epicardial Cryoablation of Myocardial Tissue Using Endocardial Thermal Isolation

MYLES EDWIN LEE, M.D.

Abstract

Surgical cure of right atrial arrhythmias may be accomplished by excision, exclusion, or cryoablation of the arrhythmogenic focus. Cryoablation may be performed epicardially without cardiopulmonary bypass but carries an operative recurrence rate of 57%. Successful cryoablation of a right atrial arrhythmia requires the reliable creation of transmural tissue necrosis. Heat transferred to the endocardium from normothermic circulating blood in the atrium or within the atrial wall may prevent full-thickness, lethal freezes. This study demonstrates that compression of myocardial tissue between an external cryoprobe and an endocardial template produces endocardial thermal isolation of the target area by displacing warm solutions from the endocardium. This is essential to achieve consistently lethal transmural freezes of −60°C. Use of this technique may reduce the high operative recurrence rate of right atrial tachycardias treated with conventional cryoablation techniques.

SUCCESSFUL SURGICAL PROCEDURES have been applied to the cure of a variety of right atrial tachycardias, including inappropriate sinus tachycardia, sinoatrial node reentrant tachycardia, and right atrial automatic and reentrant tachycardias. Epicardial cryoablation, in contrast to excisional or exclusional procedures, allows treatment of these arrhythmias without cardiopulmonary bypass but carries a 57% operative (30-day) recurrence rate (Table 1) (1–6). While it is recognized that right atrial arrhythmias may be multifocal and may masquerade as a recurrence if only one of several foci have been ablated, and that anesthetic agents may cause a sinus tachycardia indistinguishable from an atrial tachycardia, successful cryoablation of right atrial arrhythmogenic foci requires the creation of a transmural freeze, which may not be possible if warm blood, either within the atrium or within the atrial wall, transfers heat to the endocardial surface of the area being frozen.

To more precisely define the dynamics of epicardial cryoablation, we designed a study to determine those conditions necessary to consistently obtain transmural tissue temperatures of −60°C or less, which are known to be lethal to myocardial cells. The study demonstrates that transmural freezes of myocardial tissue can be achieved reliably if warm fluids are prevented from contacting its endocardial surface during cryoablation by an endocardial template positioned so as to compress the target tissue between it and the cryoprobe.

Materials and Methods

Fifteen-millimeter-square segments of sheep ventricle 2–3 mm thick were frozen with nitrous oxide (N_2O) circulating through a 15-mm flat-face cryoprobe from a Frigitronics cardiovascular sur-

---

From the Centinela Hospital Medical Center, Inglewood, California. Address reprint requests to Myles Edwin Lee, M.D., Director, Cardiothoracic Surgery, Centinela Hospital Medical Center, 555 East Hardy Street, 4th floor east, Inglewood, CA 90307.

TABLE 1

Review of Surgical Treatment of Right Atrial Arrhythmias

Rhythm	Pts.	Excision	Cryoablation	Recurrence Operative	Late
Inappropriate sinus tachycardia	3	3	—	—	—
SA node reentry	1	—	1	1	—
Right atrial tachycardia	32	26	6	4	6
Total	36	29	7	5 (4 cryo) (1 excis)	6 (2 cryo) (4 excis)

Early recurrence with cryoablation (4/7) 57.1%
Late recurrence with cryoablation (2/7) 28.6%
Early recurrence with excision (1/29) 3.4%
Late recurrence with excision (4/29) 13.8%

gical system (CCS-100, Frigitronics of Connecticut, Inc., Shelton, CT). The myocardial segments were assigned to four groups:

1. Tissue frozen in room air ($n = 19$)
2. Tissue frozen with the "endocardial" surface resting in a water bath ($n = 23$)
3. Tissue frozen resting on a wooden endocardial thermal isolator (ETI), the undersurface of which was resting in a water bath ($n = 19$)
4. Tissue frozen resting on a delrin endocardial thermal isolator (ETI), the undersurface of which was resting in a water bath ($n = 29$)

Tissue temperature was measured with a type "T" copper-constantan needle thermocouple with a temperature range of −199 to +50°C, a resolution of 1°C, and an accuracy of ±1°C. The thermocouple was placed eccentrically in each tissue segment to be as far away as possible from the cryoprobe. Circulating blood was simulated by a water bath in a stainless steel basin at physiologic temperatures. Isolation of myocardial segments from the water bath was accomplished with endocardial thermal isolators fashioned from wood or delrin (Fig. 1). The thermal isolators were held to prevent water from flowing over the top of the isolators and contacting the tissue being frozen.

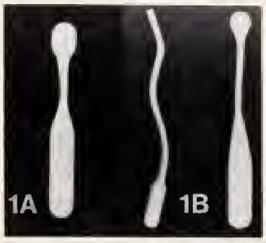


Fig. 1. Endocardial thermal isolator (1A) sculpted from wooden tongue depressor and (1B) machined from delrin rod.

Statistical Analysis. Groups were compared for initial experimental conditions (initial tissue temperature, initial probe temperature, line pressure, tissue thickness, water bath temperature) with the Kruskal-Wallis test (Table 2). The initial tissue temperature was significantly higher for tissue frozen on a wooden ETI; the initial probe temperature was significantly higher for tissue frozen in room air and on delrin; the initial line pressure was significantly less for tissue on a delrin ETI but not below 600 psi. These differences did not appear to have any clinical significance. All treatment groups were statistically equal for tissue thickness and for water bath temperature.

Success rates were compared between the groups using Fisher's exact test. The life-table method was used to obtain curves of success rate of reaching −60°C as a function of time in tissue samples subjected to cryoablation.

Results

All tissue samples frozen in room air achieved a transmural temperature of −60°C (Table 3), whereas only 26.1% of the tissue bathed in water achieved that temperature. Insulating the tissue from water with a wooden ETI resulted in only 31.6% of the tissue reaching −60°C, a result not significantly different ($p = 0.75$) from tissue in contact with warm water. Insulating tissue from water with a delrin ETI, however, allowed 96.6% of the samples to reach −60°C, a result not statistically different ($p = 0.99$) from samples frozen in room air. Comparing tissue frozen in water to that insulated with delrin, the presence of a delrin ETI decisively improved the incidence of tissue freezes to −60°C ($p <$

TABLE 2
Treatment Groups

	No ETI		Wood ETI in water bath	Delrin ETI in water bath	Kruskal-Wallis *p* value
	No water bath	Water bath			
Initial Tissue Temp (°C)					0.005
Number	19	23	19	29	
25%ile	20	24	29	25	
Median	25	30	31	30	
Mean	25.5	28.5	30.3	27.4	
75%ile	28	31	31	30	
Initial Probe Temp (°C)					0.006
Number	19	23	19	29	
25%ile	−65	−66	−67	−65	
Median	−64	−65	−66	−64	
Mean	−58.3	−61.8	−64.1	−59.6	
75%ile	−45	−59	−65	−52	
Line Pressure (PSI)					0.046
Number	19	23	19	29	
25%ile	520	550	600	560	
Median	620	600	630	570	
Mean	597	609	631	583	
75%ile	640	680	680	600	
Tissue Thickness (mm)					0.33
Number	19	23	19	29	
25%ile	2	2	2	2	
Median	2	2	2	2.5	
Mean	2.6	2.2	2.3	2.5	
75%ile	3	2.5	2.5	3	
Water Bath Temp (°C)					0.28
Number		23	19	29	
25%ile		36	36	37	
Median		37	38	38	
Mean		37.6	38.3	38.3	
75%ile		38	39	40	

0.00001). It is clear, therefore, that delrin acts as an extremely effective insulator of myocardial tissue from heat transfer by an underlying liquid at physiologic temperatures.

The time in seconds required for samples in the four groups to reach −60°C differed (Fig. 2). By 240 sec, 95% of the samples in group 1 reached −60°C, whereas only 26% of the samples in group 2 did so. Addition of a delrin ETI resulted in 87% of samples reaching −60°C in 240 sec, whereas a wooden ETI resulted in only 32% of the samples tested reaching −60°C. The percentage of tissue reaching −60°C did not increase in groups 2 or 3 despite prolongation of freezes to 360 sec, whereas in group 4, the percentage increased to 91%, and to 96.1% by 420 sec.

Fusion between the cryoprobe, the tissue being frozen, and the ETI is essential to ensure transmural freezes. When fusion between the cryoprobe and tissue was broken during the freeze, −60°C was never reached in any tissue sample (Table 3).

Discussion

Holman et al. have shown that permanent cryothermic myocardial tissue ablation requires only 2 min at −60°C (7). This study has demonstrated that if myocardial tissue can be insulated by an endocardial template at the site of freezing, this temperature can consistently be achieved and suggests that the use of an intra-atrial delrin template directly opposite an epicardial cryoprobe should be effective in reducing the recurrence rate of atrial arrhythmias treated without cardiopulmonary bypass.

Not only has this study demonstrated the effectiveness of localized endocardial thermal isolation; it also revealed important subtleties of technique, not mentioned in the clinical studies, that

TABLE 3

Comparison of Treatment Groups Undergoing Cryoablation and Effect of Breaking Fusion during Cryoablation

Treatment group	−60°C in Samples with no breaks*	−60°C in Samples with breaks	Fisher's test *p*-value
Tissue frozen in room air	19/19 = 100.0%	3/4 = 75%	0.17
Tissue frozen in water bath	6/23 = 26.1%	0/2 = 0%	0.99
Tissue on wood ETI	6/19 = 31.6%	0/6 = 0%	0.28
Tissue on delrin ETI	28/29 = 96.6%	0/7 = 0%	0.0001
Median time observed	104 sec	120 sec	

* p = 0.99 (tissue frozen in room air/tissue on delrin ETI).
p = 0.75 (tissue frozen in water bath/tissue on wood ETI).
p = 0.00001 for all other comparisons in samples with no breaks.

must be recognized to increase the chances for success:

Cryoprobe Temperature. The temperature of the cryoprobe does not reflect the transmural tissue temperature. A cryoprobe temperature of −60°C or less for 2 min does not guarantee a tissue temperature of −60°C.

Line Pressure. At a line pressure of less than 500 pounds per square inch (psi) we actually demonstrated a temperature increase of 7°C in both the cryoprobe and the tissue. Line pressures greater than 600 psi will ensure more rapid freezes with a better chance of the tissue reaching −60°C.

Breaks in Fusion. Any break in the fusion between the cryoprobe and the tissue results in an immediate rise in tissue temperature which does not decrease despite prolongation of the freeze and which was not reflected in the temperature of the cryoprobe itself. We believe this results from the instant freezing of water vapor in the air in contact with the cryoprobe and the frozen tissue. The ice that forms acts as an insulator between the cryoprobe and the tissue and could be responsible for these temperature reversals.

The tip of the cryoprobe and the target tissue, therefore, must be as dry as possible before freezing to avoid the insulating effect of ice formation between the probe and the tissue. Most important, if a break in fusion is identified, or if there is an unexplained rise in tissue temperature during a freeze, the tissue must be thawed completely and refrozen.

Pressure to Improve Fusion. Fusion can be made stronger if pressure is applied to the tissue before it is frozen. In addition to reducing tissue thickness by 0.5 mm, pressure may also reduce the possibility of autoinsulation of tissue by crystallizing tissue fluid simply by squeezing the fluid out of the target area before the freeze begins. The rigidity of delrin permits the application of such pressure.

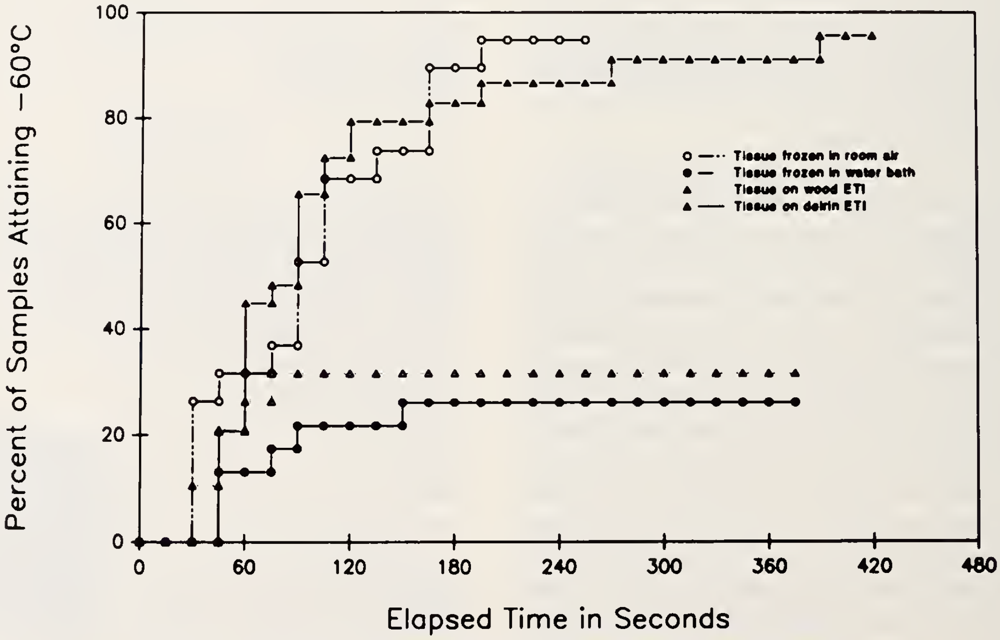


Fig. 2. Comparison of treatment groups undergoing cryoablation.

Acknowledgments

The author thanks Paul Nusser, R.N., B.S.N., and Juanita Adams, R.N., for their skilled technical assistance and Peter Christenson, Ph.D., for his statistical analysis.

References

1. Lowe JE. Surgical treatment of the Wolff-Parkinson-White syndrome and other supraventricular tachyarrhythmias. J Cardiac Surg 1986; 1:117–134.
2. Kerr CR, Klein GG, Guiraudon GM, Webb JG. Surgical therapy for sinoatrial reentrant tachycardia. PACE 1988; 11:776–783.
3. Wyndham CRC, Arnsdorf MF, Levitsky S, et al. Successful surgical excision of focal paroxysmal atrial tachycardia. Circulation 1980; 62:1365–1372.
4. Gillette PC, Wampler DG, Garson A Jr, et al. Treatment of atrial automatic tachycardia by ablation procedures. J Am Coll Cardiol 1985; 6:405–409.
5. Seals AA, Lawrie GM, Magro S, et al. Surgical treatment of right atrial focal tachycardia in adults. J Am Coll Cardiol 1988; 11:1111–1117.
6. McGuire MA, Johnson DC, Nunn GR, et al. Surgical therapy for atrial tachycardia in adults. J Am Coll Cardiol 1989; 14:1777–1782.
7. Holman WL, Ikeshita M, Douglas JM Jr, et al. Cardiac cryosurgery: effects of myocardial temperature on cryolesion size. Surgery 1983; 93:268–272.

Submitted for publication September 1991.
Revision received March 1992.

Endometriosis Causing Colon Obstruction in Two Postmenopausal Women

James G. Petros, M.D., Nicholas Spirito, M.D., and Ronald Gosshein, M.D.

Abstract

Colonic endometriosis is an uncommon lesion usually found in premenopausal women. We describe two postmenopausal women with colonic endometriosis that resulted in colonic obstruction. One of the women was receiving estrogen-replacement therapy. Colonic endometriosis in postmenopausal women may become more common as the population ages and the use of long-term estrogen-replacement therapy becomes increasingly widespread.

Endometriosis affects about 4%–17% of premenopausal women (1, 2), and the bowel is involved in 3%–34% of those cases (3, 4). Because exposure to estrogen is an important factor in the development of endometriosis, the disorder is relatively uncommon in postmenopausal women; less than 8% of cases occur in that patient population (5–7). Among older women, symptoms due to colonic endometriosis range from nonexistent to severe. We describe two postmenopausal women with colonic obstruction due to endometriosis, one of whom was receiving estrogen-replacement therapy at the time of diagnosis.

Case Reports

Case 1. A 78-year-old woman had pencil-thin stools and diarrhea for 3 weeks, along with bilateral lower abdominal cramping that preceded and was relieved by each bowel movement. Episodes of rectal discharge of mucus and bright red blood also occurred. The patient had no history of gastrointestinal disease. She was taking methyldopa and estrogen (Premarin) to prevent osteoporosis. The estrogen dosage was 0.625 mg/day, 3 weeks on therapy followed by a 1-week hiatus. Approximately 3 weeks before her diarrhea began, her serum estrone level had been 92 pg/mL (normal postmenopausal value, 30 pg/mL) and her serum estradiol level 75 pg/mL (normal postmenopausal value, 15 pg/mL).

Flexible sigmoidoscopy showed mucosal erythema, edema, and friability of the distal sigmoid colon and an area of sigmoid diverticulosis with partial obstruction approximately 20 cm from the anal verge. Biopsies revealed acute and chronic colitis with focal crypt abscesses, but no granulomas, dysplasia, or malignancy; the findings suggested inflammatory bowel disease. A limited barium-enema evaluation showed severe sigmoid diverticulosis and partial obstruction. There was no evidence of perforation. A postevacuation film obtained 24 hr later demonstrated persistence of air, fluid, and dilute barium in the colon, consistent with distal large-bowel obstruction. A pelvic computerized tomography scan revealed narrowing of the distal sigmoid lumen due to marked thickening of the bowel wall (Fig. 1). The thickened colonic wall formed a pelvic mass that was approximately 8 cm in diameter.

The patient underwent a diverting loop transverse colostomy. Postoperatively, she had

---

From the Departments of Surgery (JGP, NS) and Pathology (RG), St. Elizabeth's Hospital of Boston, and Tufts University School of Medicine, Boston, Massachusetts. Address correspondence and reprint requests to James G. Petros, M.D., Department of Surgery, St. Elizabeth's Hospital of Boston, 736 Cambridge Street, Boston, MA 02135.

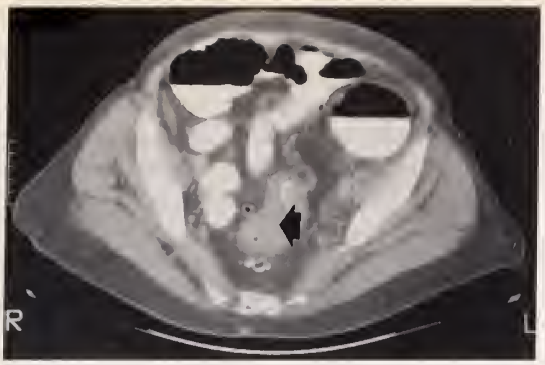


Fig. 1. Case 1. Computerized tomography scan showing marked thickening of rectosigmoid colon (arrow).

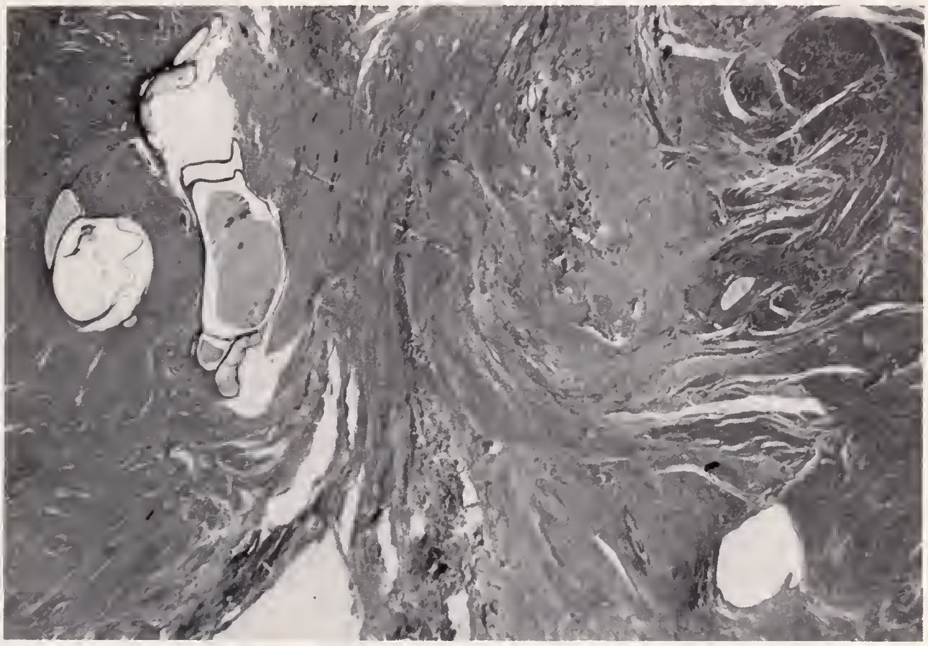


Fig. 2. Case 1. Fibromuscular hypertrophy of sigmoid muscle wall and focus of endometriosis at subserosa (H&E, original magnification ×10).

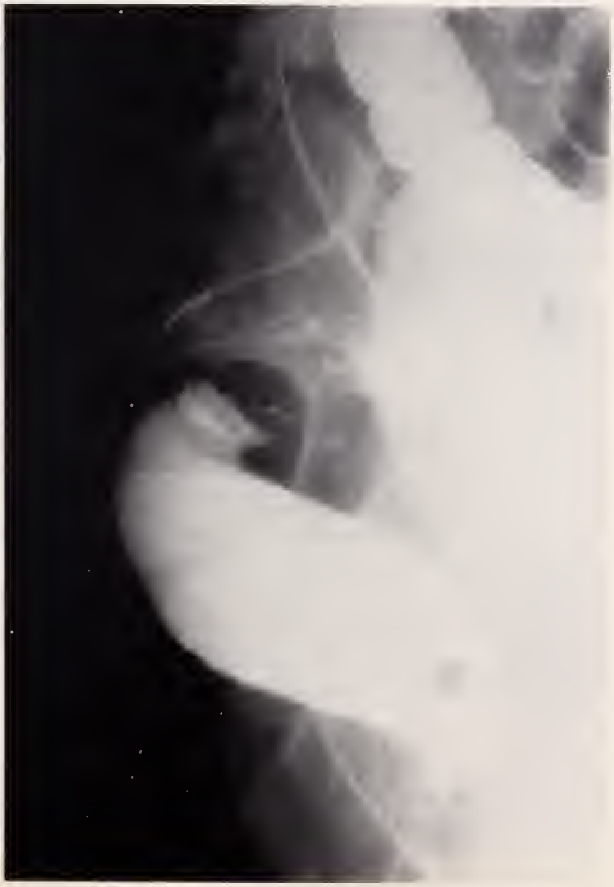


Fig. 3. Case 2. Barium-enema film showing almost complete obstruction by rectal lesion at approximately 10 cm from anal verge.

persistent diarrhea and rectal discharge of bright red blood and mucus. A *Clostridium difficile* culture yielded negative results. Flexible sigmoidoscopy through the distal limb of the colostomy showed diffuse colitis of the left and sigmoid colon with colonic spasm and aphthous ulcers. A tentative diagnosis of Crohn's colitis was made. The patient was treated with intravenous steroids, resulting in a marked decrease in stool frequency and resolution of rectal bleeding.

Six weeks later, the patient underwent exploratory laparotomy, and an 8-cm mass was discovered at the peritoneal reflection. The mass was closely adherent to the posterior bladder wall, sacral promontory, and retroperitoneal space. A low anterior resection with coloproctostomy was performed. Pathologic assessment showed a thickened colonic and rectal wall, with the colonic lumen constricted by the mass to less than 0.6 cm in diameter. Microscopy showed endometriosis of the sigmoid colon subserosa and marked muscular hypertrophy (Fig. 2).

The patient had no postoperative problems and was discharged as scheduled.

Case 2. A 67-year-old woman with no previous history of gastrointestinal problems had worsening constipation and rectal tenesmus for several months. She had stable uterine fibroids and was taking no medications. Rigid sigmoidoscopy revealed a friable lesion approximately 8 cm from the anal verge. A biopsy showed mucosal edema with underlying fibrosis. A barium-enema study demonstrated an attenuated 6-cm segment of rectum starting approximately 10 cm from the anal verge (Fig. 3) and ulcerated and distorted rectal mucosa. Subsequently, there was incomplete evacuation of barium, indicating the presence of a large-bowel obstruction, and the patient was scheduled for surgery.

During the operation, the proximal rectal mass was found to be closely adherent to the uterus. Because rectal carcinoma was suspected, the patient underwent a low anterior resection, total abdominal hysterectomy, and bilateral salpingo-oophorectomy. A pathologic evaluation showed endometriosis of the subserosa of the rectosigmoid colon and marked muscular hypertrophy in the endometriotic area.

The patient recovered from surgery uneventfully.

Discussion

Colonic endometriosis typically occurs in premenopausal women (8, 9), but postmenopausal women may also be affected. In Henricksen's series of 1000 patients with endometriosis (10), there were 37 cases of colonic endometriosis in postmenopausal women, six of whom underwent large-bowel resection.

Townell and Vanderwalt (11) observed that intestinal endometriosis may first be observed during the postmenopausal period, as it was in our patients. Williams (5) reported active endometriosis in postmenopausal women and theorized that adrenal hormones can stimulate postmenopausal endometrial activity. Rose (7), who described two cases of colonic endometriosis in postmenopausal women, postulated that endometriomas of the bowel wall are less sensitive to estrogen than those in the reproductive organs and may therefore be less likely to atrophy after menopause. Even if endometriotic lesions become inactive, the sclerotic reaction often accompanying them may continue (12). A lack of estrogen due to menopause does not necessarily lead to regression of an established endometrioma.

Exposure to estrogen can also persist after menopause. Sufficient adrenal estrogen may remain to promote endometriosis (5); peripheral conversion of androgens to estrogens may take place (13); or the patient may receive exogenous estrogen-replacement therapy. Stimulation of residual endometriosis by any of these sources of estrogen can produce symptoms (6, 13). Cust and co-workers have recommended close monitoring of postmenopausal women undergoing estrogen-replacement therapy who had experienced problems with endometriosis premenopausally (14). Most estrogen preparations produce plasma concentrations comparable to those observed during normal menstrual cycles (15). Our first patient had been taking estrogen for four years before presentation.

Lower abdominal pain with a change in bowel habits and rectal bleeding are the most common symptoms of endometriosis affecting the bowel (7–9). In postmenopausal women, the symptoms are usually episodic, with remissions and exacerbations (6). Some patients may have no symptoms (13). Others, like the two women we treated, may have severe manifestations, including conditions mimicking inflammatory bowel disease or colon cancer. Such cases of colonic endometriosis in postmenopausal women may become more common as the population ages and the use of long-term estrogen-replacement therapy becomes increasingly widespread.

Acknowledgments

The authors thank Renée J. Robillard and Robert Walkama for editorial assistance.

References

1. Gray LA. Surgical treatment of endometriosis. Clin Obstet Gynecol 1960; 3:472–491.
2. Ranney B. Etiology, prevention, and inhibition of endometriosis. Clin Obstet Gynecol 1980; 23:875–882.
3. Macafee CHG, Hardy Greer HL. Intestinal endometriosis: a report of 29 cases and a review of the literature. J Obstet Gynaecol Br Emp 1960; 67:539–555.
4. Kratzer GL, Salvati EP. Collective review of endometriosis of the colon. Am J Surg 1955; 90:866–869.
5. Williams C Jr. Endometriosis of the colon in elderly women. Ann Surg 1963; 157:974–979.
6. Kempers RD, Dockerty MB, Hunt AB, et al. Significant postmenopausal endometriosis. Surg Gynecol Obstet 1960; 111:348–356.
7. Rose TF. Complicated endometriosis of the large bowel. Med J Aust 1970; 1:891–896.
8. Meyers WC, Kelvin FM, Jones RS. Diagnosis and surgical treatment of colonic endometriosis. Arch Surg 1979; 114:169–175.
9. Graham B, Mazier WP. Diagnosis and management of endometriosis of the colon and rectum. Dis Colon Rectum 1988; 31:952–956.
10. Henricksen E. Endometriosis. Am J Surg 1955; 90:331–337.
11. Townell NH, Vanderwalt JD. Intestinal endometriosis. Postgrad Med J 1984; 60:514–517.
12. Lees DH. Severe endometriosis of the lower sigmoid colon and rectum. J Obstet Gynaecol Br Cwlth 1966; 73:272–276.
13. Gray LA. Endometriosis of the bowel: role of bowel resection, superficial excision and oophorectomy in treatment. Ann Surg 1973; 177:580–587.
14. Cust MP, Gangar KF, Hillard TC, Whitehead MI. A risk-benefit assessment of estrogen therapy in postmenopausal women. Drug Saf 1990; 5:345–358.
15. Strecker JR, Lauritzen C, Goessens L. Plasma concentrations of unconjugated and conjugated estrogens and gonadotrophins following application of various estrogen preparations after oophorectomy and in the menopause. Maturitas 1979; 1:183–190.

Submitted for publication November 1991.
Revision received February 1992.

Student Corner

Ileocecal Tuberculosis in an Asian Male Immigrant:

Case Report and Literature Review

Matthew R. Estill, M.D.

Four years after the term "tuberculosis" was coined in 1839, the first well-documented case of gastrointestinal tuberculosis was described at New York Hospital in 1843. The case report at that time depicted a disease with insidious onset and nonspecific symptoms, such as chronic abdominal pain, weight loss, low-grade fever, and anorexia:

> Wm. Henry Johnson. 40. Seaman. A month ago began to complain of want of appetite, occasional nausea, and some pain about the umbilical region, . . . pain in the abdomen became increased and has continued since, with some heat of skin and slight fever. Has had slight cough, not troubling him and not attended with loss of flesh and strength until within four weeks. Has had occasional night sweats.

Postmortem examination of this patient showed the characteristic gross pathologic changes of gastrointestinal tuberculosis: "Cadaver exceedingly emaciated . . . the peritoneum was found everywhere thickly studded with tubercles . . . the mesentery was thickened and hard, a few tubercles beneath the capsule of the liver" (1, 2).

Although gastrointestinal tuberculosis is an uncommon diagnosis in Europe and the United States today, it is nonetheless an important consideration in the differential diagnosis of chronic lower abdominal pain. As the following case demonstrates, this disease entity has a clinical picture similar to that of Crohn's disease, ileocecal lymphoma, and periappendiceal abscess (3).

Case Report

A 36-year-old Chinese-American man was admitted to The Mount Sinai Hospital with a history of chronic abdominal pain and diarrhea. Six months prior to admission, he had developed a dull, constant, nonradiating right lower quadrant pain, and three months later developed episodic, nonbloody diarrhea. The patient said he had no anorexia, nausea, vomiting, hematemesis, melena, hematochezia, constipation, or hematuria. He experienced fever to 101°, shaking chills, night sweats, and a 20-pound weight loss over the month prior to admission. The patient had no significant medical or surgical history, including no history of pulmonary or gastrointestinal disease, abnormal PPD testing, or abnormal chest x-ray. The patient had been born in Canton, China and emigrated to New York three years prior to admission. He had no history of hepatitis or parasitic disease, and his most recent travel was a trip to Hong Kong two years prior to admission. He had a 10-year history of cigarette smoking (pack) and denied alcohol or drug abuse. The patient had worked as a cook in New York City for the past three years, without known exposure to occupational or food contaminants.

On admission, the patient was noted to be a thin Oriental male with mild temporal wasting. Vital signs were blood pressure 110/70 mm Hg heart rate 72 bpm, respiratory rate 14, and temperature (oral) 98.8°. Sclera were anicteric and there was no lymphadenopathy. The abdomen was soft and slightly distended, with a 5 × 6-cm fixed, tender, nonpulsatile mass in the right lower quadrant. There was voluntary guarding over the right lower quadrant, and no rigidity, rebound, or shifting dullness. Bowel sounds were

---

Written when the author was a third-year student at the Mount Sinai School of Medicine (CUNY), originally submitted to the Department of Surgery. Address reprint requests to the author at 212 Ivy Place, 100 Wolf Road, Lebanon, NH 03766.

normal and there was no hepatosplenomegaly. Rectal examination revealed no masses or tenderness, and was guaiac positive.

Initial laboratory data included hemoglobin 10.5 g/dL, hematocrit 32.5%, white blood cell count 5,100/mm^3 with 75% neutrophils, 13% lymphocytes, 7% monocytes, 0.6% eosinophils, and 11% bands. Erythrocyte sedimentation rate was 55 mm/hr and lactate dehydrogenase 305 U/L. All other laboratory results were within normal limits. Chest x-ray on admission was normal, blood culture and sputum stain for acid-fast bacteria were negative, and stool culture was positive for *Trichuris trichura* ova.

Upper gastrointestinal series with small-bowel follow-through showed a large filling defect in the distal ileum and splaying of the cecum due to a probable right lower quadrant mesenteric mass; the typical result is shown in the figure. Colonoscopy revealed nodularity in and around the ileocecal valve, and endoscopic biopsy revealed only chronic mucosal inflammation, with no granulomata or tumor.

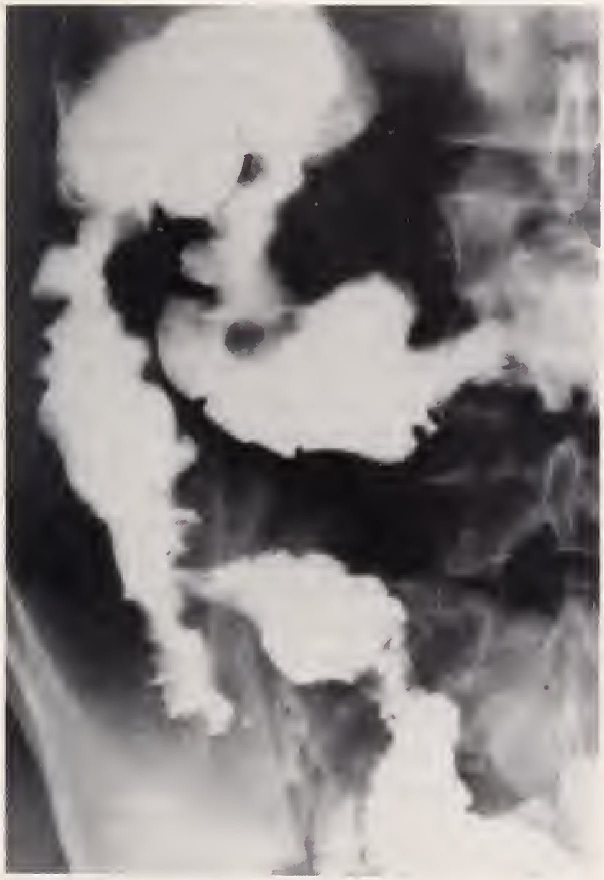


Fig. 1. Typical nonspecific findings of upper gastrointestinal series with small-bowel follow-through in ileocecal tuberculosis. There is irregular narrowing of the terminal ileum and cecum; filling defect suggestive of right lower quadrant mass found in case reported here is absent. *Reprinted* with permission from Radiology of the Colon, in: William B. Seaman, M.D., ed., Medcom Famous Teachings in Modern Medicine, New York: Medcom Inc., 1973, slide 66.

On the basis of these findings, a preliminary differential diagnosis included gastrointestinal tuberculosis, Crohn's disease, ileocecal lymphoma, or periappendiceal abscess, and empiric therapy with mebendazole, ampicillin, gentamycin, and metronidazole was begun. CT-guided needle aspiration of the abdominal mass the following day drained straw-colored fluid which grew out *Bacteriodes fragilis.* In order to make a definitive tissue diagnosis, an exploratory laparotomy was performed two days later. It showed multiple "miliary" nodules throughout the peritoneal cavity, with deposits on the mesentery, cecal, and ileal wall. Frozen-section biopsy revealed necrotic granuloma and chronic inflammatory infiltrate in the cecum "suspicious for tuberculosis"; however, acid-fast staining of this tissue was negative. The mass was noted to be a phlegmon composed of multiple large mesenteric nodes. The patient was treated with rifampin, isoniazide, and ethambutol postoperatively, with plans for a nine-month antituberculous regimen. On postoperative day six the patient was discharged in stable condition. Two weeks after discharge, tissue culture from the omental biopsy taken at laparotomy grew out *Mycobacterium tuberculosis.*

Discussion and Literature Review

Incidence. In the first half of this century, gastrointestinal tuberculosis steadily increased in incidence in the United States and Europe. However, with the introduction of antituberculous chemotherapy after World War II and the initiation of routine tuberculin testing of dairy herds in the United States, the reported incidence of gastrointestinal tuberculosis fell (1). In addition, the routine pasteurization of milk in western Europe and the United States eliminated, among other pathogens, *Mycobacterium bovis,* an acid-fast bacillus that has been postulated to be the causative organism of primary gastrointestinal tuberculosis (4). Following the classic description of granulomatous regional enteritis (Crohn's disease) as a distinct pathological entity by Crohn, Oppenheimer, and Ginzburg in 1932 (5), the incidence of confirmed tuberculous gastroenteritis fell dramatically in the United States (6). In fact, it is difficult to interpret the literature prior to 1932, since many cases of regional enteritis had most likely been classified as tuberculosis prior to Crohn and colleagues' classic report (7).

In the last two decades, gastrointestinal tuberculosis had a reported incidence of 0.1% to 0.9% of all reported cases of tuberculosis in the United States (8). In the largest recent survey of North American cases, Sherman and colleagues reported only 36 cases (0.85%) of gastrointestinal involvement among 4,222 consecutive patients diagnosed with tuberculosis over the 17-year period from 1960 to 1979 (9). Weir and colleagues reported only four cases of gastrointestinal involvement in a survey of 36 cases of extrapulmonary disease (3).

However, the recent reemergence of tuberculosis in large numbers in urban areas warrants an increased awareness of the signs and symptoms of gastrointestinal tuberculosis (10). In addition, tuberculosis remains a major public health problem of epidemic proportions in most developing countries, especially in southeast Asia and the Middle East, where as many as 1.5% of all cases of tuberculosis in Sri Lanka and Iran exhibit gastrointestinal involvement (11).

As reported in several other large North American and European review series, this disease commonly occurs in the fourth decade, has a 3:1 male:female ratio, and occurs in highest frequency in the Asian male immigrant population (12). Interestingly, in several studies from southeastern Asia the male–female distribution is reversed, which may be explained by the large number of male immigrants to the West who have lived in overcrowded conditions and may thus have higher exposure rates (13).

Signs and Symptoms. The most common signs and symptoms of gastrointestinal tuberculosis are vague lower abdominal pain of several months' duration, weight loss, anorexia, and fever, with or without an abdominal mass, ascites, and night sweats. Because of its insidious, nonspecific symptoms, this disease is often misdiagnosed, and there is wide variation in reported symptomatology. However, the signs and symptoms in the case report above are by no means atypical and have been widely reported in other larger series. In a series of 30 cases by Wells and colleagues (13), chronic abdominal pain occurred in 22 of the 30 patients in the study (71%), and weight loss in 24 (80%), while signs included fever in 25 (82%), abdominal tenderness in 16 (52%), ascites in 14 (48%), and a palpable abdominal mass in only 6 (20%). In Sherman and colleagues' series of 36 patients (9), 26 (72%) had weight loss, 21 (58%) anorexia, 13 (36%) chronic abdominal pain, 13 (36%) pulmonary symptoms, and 9 (25%) nausea and vomiting, while 26 (72%) had fever and 21 (55%) had abdominal tenderness.

In both of these studies there was a long latency period between onset of symptoms and hospitalization, Wells and colleagues (13) reporting onset of symptoms (chronic abdominal pain, weight loss, and fever) from four weeks to six months prior to admission. In addition, Vyravanathan and colleagues reported that in a series of 35 cases in Sri Lanka, the onset of symptoms (fever, abdominal pain, anorexia, and weight loss) was more than one week prior to hospitalization in all cases, and that in 85% they had been present for more than one month prior to hospitalization. In contrast to the abdominal mass in the patient described above, only 19 of 35 patients (54%) in Vyravanathan's study had an abdominal mass (14). Schulze and colleagues (4) found that 7 of 13 patients with abdominal tuberculosis had a history of previous tuberculosis or exposure to tuberculosis, which supports the hypothesis that gastrointestinal tuberculosis primarily arises via secondary spread from a tuberculous focus elsewhere in the body. However, since the symptoms of this disease are insidious, it is difficult to precisely determine the latency period from initial infection to the onset of symptoms.

Clinical Diagnosis. Undoubtedly, the anatomic location of the infection within the gastrointestinal tract influences the clinical symptomatology. Weight loss and nutritional deficiencies might be expected with terminal ileal involvement, while mesenteric and omental involvement could potentially produce obstructive symptoms due to the mass effect of enlarged, granulomatous peritoneal lymph nodes.

Noninvasive Testing. In reviewing the sparse clinical literature on this subject, it is difficult to determine the number of actual cases of gastrointestinal tuberculosis excluded from retrospective studies because of the difficulty in documenting *Mycobacterium* by culture or acid-fast stain. In fact, in the absence of either positive AFB staining or positive cultures from regional biopsies, it can be extremely difficult to distinguish tuberculosis from Crohn's disease on the basis of microscopic histopathologic changes alone (15).

Gastrointestinal tuberculosis most often affects the ileocecal region, which has been postulated to be due to the high concentration of lymphoid tissue in and around the appendix (16). Involvement of stomach, proximal small bowel, and distal colon is rare. Since the typical histopathologic pattern of epithelioid granulomas, caseating necrosis, and chronic inflammatory infiltrate in the ileocecal region reflects nonspecific change, acid-fast staining and culture for mycobacteria in tissue biopsy are most often used to establish a definitive diagnosis. However, as seen in the case above, acid-fast staining of peritoneal aspirate, colonoscopic biopsy, and even surgically acquired biopsies can give false negative results. However, others have shown that the diagnostic yield of positive AFB staining is twice as high in surgical samples as it is in colonoscopic biopsies. It has been estimated that at least 100,000 organisms per milligram of tissue are needed before acid-fast stains are positive (12). In the absence of positive acid-fast staining, one must often wait for the results of tissue culture (up to 8 weeks for the slow-growing mycobacterial strains) to make a definitive diagnosis of gastrointestinal tuberculosis. However, empiric antituberculous pharmacotherapy has been employed on the basis of "suggestive" findings from tissue biopsy, even in the absence of positive acid-fast stains.

It has been theorized that gastrointestinal tuberculosis can result from either direct invasion of the mucosal wall by ingested mycobacteria, or as a secondary bacteremic or lymphangitic spread from dormant or active tuberculous infection elsewhere (12). Since the reported incidence of primary gastrointestinal tuberculosis is rare, a search for infectious foci elsewhere is often performed in all patients in which gastrointestinal tuberculosis is suspected. In other words, in patients with chronic unexplained abdominal pain with or without a mass, fever, or weight loss, and especially an Asian male immigrant, PPD skin testing, chest x-ray, and acid-fast staining of sputum have been advocated (15). As the case above illustrates, one must often wait several weeks for positive tissue-culture results, which makes early diagnosis by AFB staining, skin testing, or chest x-ray helpful (13). However, negative skin testing with full-strength PPD has been reported in 35% of cases proven by AFB staining or positive culture by Wells and colleagues (13), while Sherman and colleagues (9) reported a 28% rate (7 of 25 patients) of negative PPD skin tests. Normal chest x-rays have been reported in 50% and 68%, respectively, in two other large series of patients with gastrointestinal tuberculosis (1, 2). While the upper gastrointestinal series in the case report above revealed an abnormality in the ileocecal region, this procedure yields nonspecific findings and is inconclusive in establishing the diagnosis of gastrointestinal tuberculosis (13).

Positive stool guaiac testing, while seen in six out of eight cases by Schulze and colleagues (4), obviously is not specific for gastrointestinal tuberculosis and has a low positive predictive value, given the low prevalence of this disease. The most sensitive hematologic abnormality has been shown to be an elevated erythrocyte sedimentation rate, Wells and colleagues (13) finding that in 22 of 26 patients (85%) it was elevated greater than 20 mm/hr and that in 16 of 26 (62%), greater than 40 mm/hr. However, the erythrocyte sedimentation rate is a nonspecific indicator of chronic disease and can be elevated in other gastrointestinal diseases, such as colon cancer and the chronic inflammatory conditions like Crohn's disease and ulcerative colitis. Most series have shown the white blood cell and lymphocyte counts to have a low sensitivity and specificity for gastrointestinal tuberculosis, Wells and colleagues (13) reporting a white blood cell count over 11,000 in only 3 of 26 patients (11%). Finally, aspiration of gastric contents with staining for acid-fast bacilli and culture has been reported to have an extremely low diagnostic yield for tuberculosis. Schulze and colleagues (4) reported a sensitivity of 14% for acid-fast staining and culture of gastric aspirate samples in patients with gastrointestinal tuberculosis, three of the four positive samples coming from patients who were found to have gastric involvement by tuberculous bacilli.

Invasive Modalities of Testing. Given the poor diagnostic yield of noninvasive testing for gastrointestinal tuberculosis, which invasive modality is most useful in diagnosis? While colonoscopy has been increasingly utilized as a diagnostic procedure in patients with chronic abdominal pain with or without a mass, its diagnostic yield for gastrointestinal tuberculosis has been reported to be lower than that of surgically acquired specimens (12). Colonoscopic diagnosis has been made on the basis of caseation necrosis with granulomas, staining for acid-fast bacilli, and positive growth of mycobacteria on culture (17). However, caseation necrosis is not invariably present in mucosal biopsy samples and is not specific for tuberculosis, and acid-fast bacilli are found more commonly in regional lymph nodes than in the mucosal tissue obtained by colonoscopy. In addition, incidental acid-fast organisms from the colonic lumen can potentially yield false positive results (12). Although peritoneal fluid aspiration in the presence of ascites has been reported to be helpful in making a diagnosis, it has been reported that up to 83% of paracentesis aspirates have been negative for acid-fast staining and show no growth in culture (18). Other studies have shown the diagnostic yield of needle aspiration of ascitic fluid to be extremely low, even in the presence of gastrointestinal tuberculosis subsequently documented by laparotomy or laparoscopy (19). In addition, most studies have reported an incidence of clinically demonstrable ascites in gastrointestinal tuberculosis of approximately 50%, Vyravanathan and colleagues reporting that 16 of 35 (45%) had ascites on initial physical examination (14).

Prior to the advent of laparoscopy, surgical diagnosis had traditionally been accomplished with laparotomy, which allowed for adequate sampling of peritoneal lymph nodes, visual inspection of the mesentery, and exploration of any mass present. Laparoscopy with targeted peritoneal and mesenteric biopsy has recently been advocated as the diagnostic procedure of choice in patients with gastrointestinal tuberculosis. In a 1979 study of 11 cases proven by acid-fast stain or culture to be gastrointestinal tuberculosis, Wolfe and colleagues showed that laparoscopically directed biopsy of affected nodes positively identified 8 cases (20). In a 1978 study, Udwadia and colleagues obtained 29 tuberculous lesions in 384 laparoscopies performed in Bombay, India. All of the patients in this study had a history of chronic abdominal pain, weight loss, or fever, and all other standard diagnostic tests, including paracentesis with culture and acid-fast staining, had been negative for tuberculous bacilli (21). Direct laparoscopic visualization of the peritoneum, mesentery, and affected bowel often reveals a characteristic pattern of confluent or scattered miliary nodules of uniform size, adhesions between affected bowel loops and the abdominal wall (22).

Empiric Therapy. Empiric chemotherapy consisting of isoniazide and two other antituberculous agents, most commonly ethambutol and rifampin, has been used successfully on the basis of positive acid-fast stains or as empiric therapy until culture results are known. In several large series, patients have responded to nine months of antituberculous therapy without sequelae (1–3, 13).

[Because of the rise in multidrug-resistant tuberculosis in New York City, the New York City Department of Health now recommends initiating therapy of tuberculosis with four drugs—INH, rifampin, pyrazinanide, and ethambutol—until results of testing are available (Jack Adler, M.D., personal communication).]

References

1. Dineen P, et al. Tuberculous peritonitis: 43 years' experience in diagnosis and treatment. Ann Surg 1976; 184: 717–725.
2. Addison N, et al. Abdominal tuberculosis—a disease revisited. Ann R Coll Surg Eng 1983; 65:105–112.
3. Weir M, et al. Extrapulmonary tuberculosis: experience at a community hospital and review of literature. Am J Med 1985; 79:467–474.
4. Schulze K. Intestinal tuberculosis: experience at a Canadian teaching institution. Am J Med 1977; 63:735–742.
5. Crohn B, et al. Regional ileitis: a pathological and clinical entity. JAMA 1932; 70:158–164.
6. Crohn B, et al. Primary ileocecal tuberculosis. NY State J Med 1940; 70:158–162.
7. Paustian F, Bockus H. So-called primary ulcerohypertrophic ileocecal tuberculosis. Am J Med 1959; 27:509–518.
8. Palmer K, et al. Abdominal tuberculosis in urban Britain—a common disease. GUT 1985; 26:1296–1305.
9. Sherman S, et al. Tuberculous enteritis and peritonitis: report of 35 general hospital cases. Arch Intern Med 1980; 140:506–517.
10. Pitichinik A, et al. Tuberculosis, atypical mycobacteriosis, and the acquired immunodeficiency syndrome among Haitian and non-Haitian patients in South Florida. Ann Intern Med 1984; 101:641–647.
11. Borhanmanesh F, et al. Tuberculous peritonitis: prospective study of 32 cases in Iran. Ann Intern Med 1972; 76:567–572.
12. Paustian F, et al. Intestinal tuberculosis. In: Berk J, ed. Bockus Gastroenterology, 4th ed., vol. 3. Philadelphia: W. B. Saunders, 1985:2018–2036.
13. Wells A, et al. Abdominal tuberculosis: still a problem today. J R Soc Med 1986; 79:149–159.
14. Vyravanathan S, et al. Tuberculous peritonitis: a review of thirty-five cases. Postgrad Med J 1980; 56:649–651.
15. Graeme-Cook F. A 17-year-old Cambodian girl with recurrent abdominal pain and a tender mass in the right lower quadrant. Case Records of the Massachusetts General Hospital. N Engl J Med 1991; 325(18):1295–1302.
16. Findlay J. Tuberculosis of the gastrointestinal tract. In: Allen R, ed. Inflammatory Bowel Disease. Edinburgh: Churchill Livingstone, 1983:562–571.
17. Koo J, et al. The value of colonoscopy in the diagnosis of ileocecal tuberculosis. Endoscopy 1982; 14:48–50.
18. Singh M, et al. Tuberculous peritonitis. An evaluation of the pathogenetic mechanisms, diagnostic procedures, and therapeutic measures. N Engl J Med 1969; 281: 1091–1094.
19. Bastani B, et al. Tuberculous peritonitis—report of 30 cases and review of the literature. Q J Med 1985; 56: 549–557.
20. Wolfe J, et al. Tuberculous peritonitis and the role of diagnostic laparoscopy. Lancet 1979; 1:852–854.
21. Udwadia J. Peritoneoscopy in the diagnosis of abdominal tuberculosis. Indian J Surg 1978; 40:91–95.
22. Geake T, et al. Peritoneoscopy in the diagnosis of tuberculous peritonitis. Gastrointest Endosc 1981; 27:66–70.

Submitted for publication November 1991.
Revision received February 1992.

When you don't know all
the medicines your patients are taking,
protecting their health
is a crap shoot.

Counseling patients about all their medicines improves their odds of getting well and staying well.

EVERYONE WINS WHEN YOU TALK

Send me a *free* Medicine Counseling Kit.

Name

Organization

Street

City State Zip

Mail to:
National Council on Patient Information
and Education
666 Eleventh Street, NW, Suite 810
Washington, DC 20001
To fax your request -- (202) 638-0773

this publication is available in microform

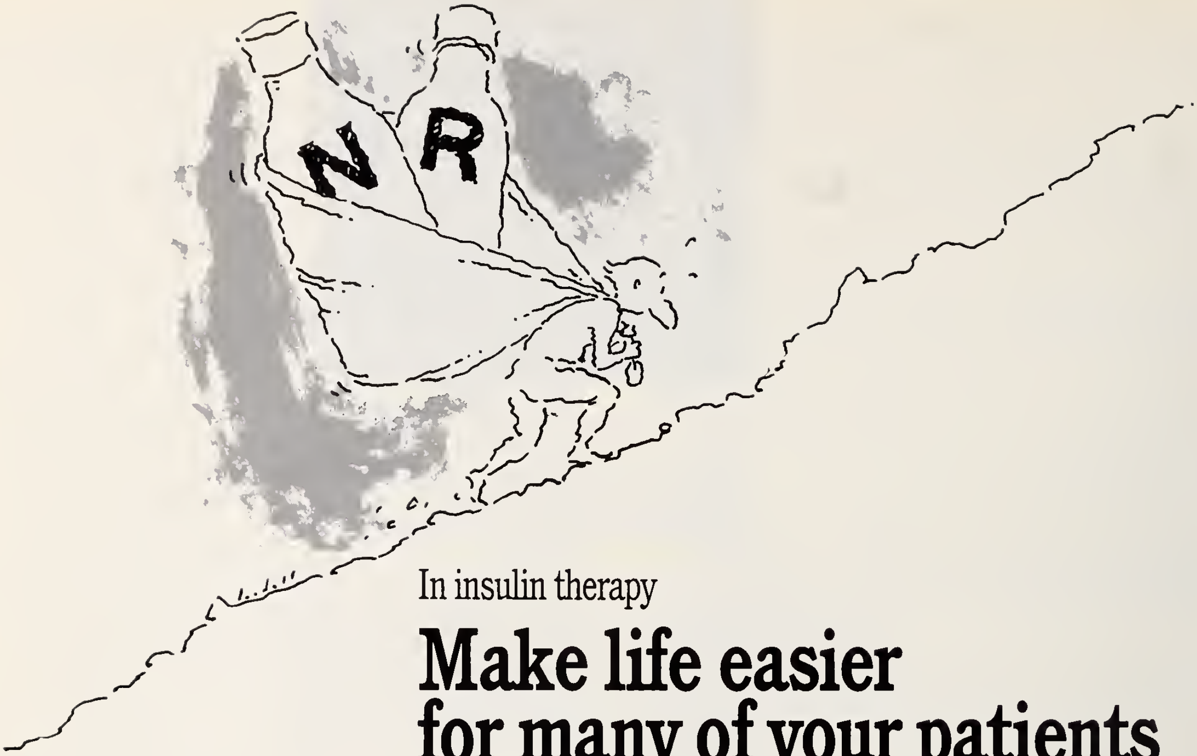
Please send me additional information.

University Microfilms International

300 North Zeeb Road
Dept. P.R.
Ann Arbor, MI 48106
U.S.A.

18 Bedford Row
Dept. P.R.
London, WC1R 4EJ
England

Name ______
Institution ______
Street ______
City ______
State ______ Zip ______



In insulin therapy

Make life easier for many of your patients

Humulin 70/30. Convenient and simple to administer. No more mixing. No more mixing errors. All of which makes living with diabetes a little easier for patients. And compliance a lot easier to achieve.

Humulin® 70/30

70% human insulin isophane suspension
30% human insulin injection
(recombinant DNA origin)

The patient-friendly premix

Global Excellence in Diabetes Care
Eli Lilly and Company
Indianapolis, Indiana
46285

WARNING: Any change of insulin should be made cautiously and only under medical supervision.

HI-7905-B-249327 ©1992, ELI LILLY AND COMPANY

Instructions for Authors

THE MOUNT SINAI JOURNAL OF MEDICINE, established in 1934 as *The Journal of the Mount Sinai Hospital*, is a peer-reviewed general medical journal. It is indexed or abstracted in *Index Medicus, Current Contents, Science Citation Index, Hospital Literature Index, International Nursing Index, Excerpta Medica, Chemical Abstracts, Biological Abstracts,* and major databases. Theoretical and basic science articles for the clinician, reviews, reports of clinical or research experience, articles on any subject affecting the practice of medicine, short notes in any specialty, book reviews, letters, and articles by medical students are welcome. The *Journal* is a participant in the Agreement on Uniform Requirements for Manuscripts Submitted to Biomedical Journals. The *Journal* aims at responding to authors of manuscripts within 8 weeks of date of submission.

Communications. Address correspondence on manuscripts review to: Managing Editor, *The Mount Sinai Journal of Medicine,* Box 1094, 50 E. 98th Street, 1E, New York, NY 10029-6574. Phone: (212) 241-6108; FAX: (212) 722-6386.

Copyright. The publisher reserves copyright and renewal rights on all published material, and such material may not be published elsewhere without written permission of the publisher. When submitting a manuscript, include the following assignment of copyright in your cover letter: **In consideration of The Mount Sinai Journal of Medicine (MSJM) taking action and reviewing and editing my (our) submission, the author(s) undersigned hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership to The Mount Sinai Journal of Medicine in the event that such work is published by MSJM.** Each author must sign the statement.

Manuscript Preparation. Submit manuscripts in triplicate, including three sets of illustrations. Manuscripts must be typewritten, **double-spaced throughout (including references, legends, and tables)**, on one side of 8½ × 11-in. white bond paper, with generous margins. Number pages consecutively. Begin a new page for title page, abstract, first page of text, references, each table, and legends. **Computer-generated typescripts** must be double-spaced and must be easy for editors and typesetters to read. **Computer tapes or disks:** consult managing editor.

Title Page. On the title page, on separate lines (double-spaced), include (1) title of article, phrased as concisely as possible; (2) name of each author, including first name and degree; (3) name and address of departments and institutions from which the work originated; (4) acknowledgments of sources of support; (5) name, place, and date of any conferences at which paper has been delivered; (6) the statement **Address reprint requests to . . . ,** giving full name and address, with zip code, of the appropriate author.

Abstract. Original articles should include an abstract, which should be a single paragraph not exceeding 150 words. State the purposes; basic approach or methods; main findings; principal conclusions. Emphasize new and important aspects. When an abstract is provided, a summary at the end is not necessary. Reviews and other articles without an abstract should conclude with a short summary.

Key Words. Supply 3 to 10 key words or phrases at the bottom of the title page, for use in indexing the article. Use Medical Subject Headings from *Index Medicus.*

Writing Style and Abbreviations. Authors should aim for conciseness and clarity without repetition. Avoid medical jargon, abbreviated phrasing, and obscure or newly coined abbreviations. Define all abbreviations on first use, except those for standard, universally recognized units of measurement and statistical terms. Spell out such terms as white blood cell, hematocrit, superior vena cava.

Units of Measurement. Use SI units, giving range or interval of normal values.

Editing. All papers will be edited to enhance conciseness and clarity without altering meaning and to insure conformity with journal style. The author is responsible for all statements in the article.

Illustrations. Photographs, line drawings, graphs, and charts should increase understanding of the text. Line drawings, graphs, and charts should be professionally prepared. All illustrations should be submitted in triplicate as sharp, high-contrast glossy prints. Photomicrographs must be 5⅛ in. wide. On the back of each print affix a gum label with the number of the figure (numbered consecutively in arabic numerals as cited in text), title of manuscript, name of senior author, and the word "top" with an arrow indicating top edge. Illustrations which are to appear together should be mounted accordingly as plates and should correspond to each other in size. Protect the prints by enclosing them in heavy cardboard; do not use paper clips. Photographs of patients can be published only if a copy of the permission is submitted with the photograph. Eliminate names of patients and hospital numbers from x-rays.

Legends. Legends should not duplicate the text. Double space. Number each legend to match the illustration it accompanies. Illustrations mounted as plates to appear together should be accompanied by a single legend identifying the separate elements by position ("upper left," and so on) or by letters. If letters are used in the legend, a corresponding letter must appear on the print itself. Identify all abbreviations. Indicate magnification and stain for photomicrographs.

Tables. Each table should be typed on a separate page, double spaced. Number tables consecutively as they appear in text, using arabic numerals ("Table 1"). Give each table a brief title (for example, "Effect of DMSO on Rats") and type it on a separate line. If abbreviations or symbols are used in the table, identify them in a footnote.

References. When citing references in your text, the first work cited is numbered 1, and succeeding references are numbered in sequence; enclose the citation number in parentheses and place it before any punctuation: **Cytomegalovirus (1), steroids (2), and recreational drugs (3) have all been implicated (4).** The reference list includes only works cited in the text, numbered in the order in which they are cited. Type, double spaced, following the general arrangement and punctuation style in the examples below (journal title abbreviations conform to *Index Medicus* style):

1. Nadelman RB, Wormser GP. A clinical approach to Lyme disease. Mt Sinai J Med 1990;57:144–156.

2. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. N Engl J Med 1991;324:424–428.

When a manuscript is accepted for publication, you will be asked to confirm the accuracy of all references.

Proofs. Proofs are sent to the corresponding author from the printer. Stylistic changes which do not alter meaning should not be made at this stage. Because of increased printing charges, the cost of excessive author's alterations beyond routine correction of typographical errors and major errors in data may be billed to the author. Proofs should be corrected and returned to the editorial office within 48 hours.

Reprint Orders. A reprint order form is sent to the corresponding author with proofs; return it to the editorial office.

THE
MOUNT SINAI JOURNAL
OF MEDICINE

Forthcoming:

Grand Rounds

Narrative and Medicine
Oliver Sacks
Albert Einstein College of Medicine

Osteoporosis: On the Verge of Rational Effective Therapy?
John Paul Bilezikian
Columbia University College of Physicians and Surgeons

The Global Impact of Penicillin: Then and Now
Gene H. Stollerman
Boston University School of Medicine

Obscure Gastrointestinal Bleeding
Blair Lewis
Mount Sinai School of Medicine

Pharmacology of Antiinflammatory Agents: A New Paradigm
Bruce N. Cronstein
New York University School of Medicine

Asthma as an Inflammatory Disease
Kirk Sperber
Mount Sinai School of Medicine

Theme Issues

OCTOBER 1992

Human Cancer: From Precurable to Curable
Editors: Max Sung and Takao Ohnuma
plus general articles

NOVEMBER 1992

Urban Community Medicine: In Honor of Kurt Deuschle
Editors: Alan Silver and David Rose

JANUARY 1993

Issues in Medical Ethics:
1. The Physician-Patient Contract
2. Transplantation
Editors: Rosamond Rhodes and Daniel A. Moros

MAY 1993

Quality Assurance
Editor: Suzanne Kramer

OCTOBER 1993

Issues in Medical Care for Children
Editor: Richard Saphir

LEVY LIBRARY, MT. SINAI MEDICAL CENTER

Available now:

Toward the Conquest of Pain
Allan P. Reed, editor
volume 58, no. 3, May 1991
84 pages $15

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society
Eugene W. Straus, editor
volume 59, no. 2, March 1992
96 pages $15

Modern Management of Malignant Melanoma
Michail Shafir, editor
volume 59, no. 3, May 1992
86 pages $15

Subscriptions for 1992: $60 individuals, U.S. and Canada; $65 all libraries; $70 individuals outside U.S. and Canada.

To order subscriptions or copies of back issues ($15 each), please send check, payable to The Mount Sinai Journal of Medicine, to the Journal at Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574; counter sales at Suite 1E, 50 E. 98th Street, New York, NY (phone: 212 241-6108; FAX: 212 722-6386).

MOUNT SINAI SCHOOL OF MEDICINE
OF THE CITY UNIVERSITY OF NEW YORK

THE MOUNT SINAI JOURNAL OF MEDICINE

OLUME 59 NUMBER 5 OCTOBER 1992

OCT 22 1992

Human Cancer: From "Precurable" to Curable

Commemorating the 65th Birthday of Dr. James F. Holland

Max W. Sung and Takao Ohnuma, editors

From the Department of Neoplastic Diseases and the
Derald H. Ruttenberg Cancer Center
Mount Sinai Medical Center

plus

general articles on tamoxifen and endometrial cancer, CB3717, combination-mode therapy of neurofibrosarcoma, new treatment for chemotherapy-induced emesis

Veterans Affairs

BETH
ISRAEL
MEDICAL
CENTER

THE MOUNT SINAI JOURNAL OF MEDICINE

The Mount Sinai Journal of Medicine is published by The Mount Sinai Medical Center of New York and has the following affiliates: Beth Israel Medical Center, New York; Bronx Veterans Affairs Medical Center, New York; and Elmhurst Hospital Center, New York.

Editor-in-Chief

Sherman Kupfer, M.D.

Editor Emeritus

Lester R. Tuchman, M.D.

Associate Editors

Harriet S. Gilbert, M.D. Julius Wolf, M.D.

Managing Editor

Claire Sotnick

Business and Production Assistant

Claudia N. Melendez

Assistant Editors

Stephen G. Baum, M.D.
David H. Bechhofer, Ph.D.
Constanin A. Bona, M.D., Ph.D.
Edward J. Bottone, Ph.D.
Jurgen Brosius, Ph.D.
Lewis Burrows, M.D.
Joseph S. Eisenman, Ph.D.
Adrienne M. Fleckman, M.D.
Richard A. Frieden, M.D.
Steven Fruchtman, M.D.
Paul L. Gilbert, M.D.
James H. Godbold, Ph.D.
Richard S. Haber, M.D.
Noam Harpaz, M.D.
Dennis P. Healy, Ph.D.
Tomas Heimann, M.D.
Barry W. Jaffin, M.D.
Andrew S. Kaplan, D.D.S.
Samuel Kenan, M.D.
Suzanne Carter Kramer, M.Sc.
Mark G. Lebwohl, M.D.
Kenneth Lieberman, M.D.
Charles Lockwood, M.D.
Steven Markowitz, M.D.
Bernard Mehl, D.P.S.
Myron Miller, M.D.
Edward Raab, M.D.
Allan Reed, M.D.
Allan E. Rubenstein, M.D.
David B. Sachar, M.D.
Henry Sacks, M.D.
Robert Safirstein, M.D.
Ira Sanders, M.D.
Martin H. Savitz, M.D.
Clyde B. Schechter, M.D.
Michael Serby, M.D.
Phyllis Shaw, Ph.D.
George Silvay, M.D.
Barry D. Stimmel, M.D.
Nelson Stone, M.D.
Max Sung, M.D.
Carl Teplitz, M.D.
Rein Tideiksaar, Ph.D.
Richard P. Wedeen, M.D.
Michael F. Wesson, M.D.

Editorial Board

Barry Freedman, M.B.A.
Richard Gorlin, M.D.
Nathan Kase, M.D.
Panayotis G. Katsoyannis, Ph.D.
Charles K. McSherry, M.D.
Jack G. Rabinowitz, M.D.
John W. Rowe, M.D.
Alan L. Schiller, M.D.
Alan L. Silver, M.D.
Alvin S. Teirstein, M.D.
Rosalyn S. Yalow, Ph.D.

The Mount Sinai Journal of Medicine (ISSN No. 0027-2507; USPS 284-860) is published 6 times a year in January, March, May, September, October, and November in one indexed volume by the Committee on Medical Education and Publications (Owner), The Mount Sinai Hospital, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Subscription price: individuals, U.S. and Canada, $60 per year; all libraries, $65; individuals outside the U.S. and Canada, $70. Single copies, $15. New subscriptions begin with the first issue of the calendar year. Send notice of change of address 60 days before the change is effective. Second-class postage paid at New York, NY and at additional mailing offices. POSTMASTER: Send address changes to The Mount Sinai Journal of Medicine, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Copyright 1992 The Mount Sinai Hospital.

THE
MOUNT SINAI JOURNAL
OF MEDICINE

Volume 59
Number 5
October 1992

CONTENTS

Human Cancer: From "Precurable" to Curable—Commemorating the 65th Birthday of Dr. James F. Holland *Max W. Sung and Takao Ohnuma, editors*

Introduction *Takao Ohnuma* 373

James F. Holland: A Brief Synopsis of His Career 374

About James F. Holland *Joseph H. Burchenal* 375

Reminiscences 377

The Interface of Science and Medicine *Joshua Lederberg* 380

Cancer Cures Yesterday, Today, and Tomorrow *Jean Bernard* 384

Protein Kinase C: Implications for Cancer Prevention and Treatment *I. Bernard Weinstein* 387

Enzymes of the Thymidylate Cycle as Targets for Chemotherapeutic Agents: Mechanisms of Resistance *Joseph R. Bertino, Wei Wei Li, James Lin, Tanya Trippett, Erdem Goker, Barry Schweitzer, and Debabrata Banerjee* 391

Impact of Cytogenetics and Molecular Genetics on Leukemia Therapy *Emil J Freireich* 396

Consistent Genetic Abnormalities in Human Cancers as Targets for Selective Therapies *Bayard D. Clarkson* 400

Advances in Treatment for Hodgkin's Disease *Peter H. Wiernik* 405

The Evolution of Curative Cancer Chemotherapy *Emil Frei III* 408

The Past—Prelude to the Future *Ezra M. Greenspan* 412

Successful Successive Studies in Leukemia and Breast Cancer *James F. Holland* 414

GENERAL ARTICLES AND CASE REPORTS

Tamoxifen and Endometrial Cancer *Rudy A. Segna, Peter R. Dottino, Liane Deligdisch, and Carmel J. Cohen* 416

continued

Volume 59
Number 5
October 1992

CONTENTS *continued*

N^{10}-propargyl–5,8-dideazafolic acid (CB3717): Inhibitory Effects on Human Leukemia Cell Lines Resistant to Methotrexate or Trimetrexate *Yuruzu Takemura and Takao Ohnuma* 419

Peripancreatic Lymph Node Gastrinoma in a Patient with Zollinger-Ellison Syndrome *Hirshel Kahn and Jenny Waxman* 425

Treatment of a Large High-Grade Neurofibrosarcoma with Concomitant Vinblastine, Doxorubicin, and Radiotherapy *Howard W. Bruckner, Mayer Gorbaty, Roberto Lipsztein, Giselle Kranjac, and Mark G. Lebwohl* 429

THE FORMULARY

Serotonin Antagonists: Treatment of Chemotherapy-Induced Emesis *Anthony Barisano, Bernard Mehl, and Keith Bradbury* 433

Index to Advertisers

Critical Care America page 438

Human Cancer: From "Precurable" To Curable

Introduction

Takao Ohnuma, M.D., Ph.D.

The symposium "Human Cancer: From 'Precurable' to Curable" was held on May 29, 1990 to commemorate the 65th birthday of James F. Holland. This was one of the most joyous occasions for the Derald H. Ruttenberg Cancer Center and the Department of Neoplastic Diseases at the Mount Sinai Medical Center.

James Holland has devoted his entire career to the treatment of cancer and leukemia. His accomplishments are too numerous to mention, but if I have to choose one, I would pick his demonstration that acute leukemia in children can now be cured. Many of us can recall being taught in medical school that leukemia was an incurable disease. Indeed, in 1956, when the Cancer and Leukemia Group B (CALGB) activated its first chemotherapy protocol for acute lymphoblastic leukemia (ALL) in children, all patients with the disease died within one and a half years. During Dr. Holland's chairmanship of the CALGB, he instituted progressive refinements of combination chemotherapy, producing corresponding increases in survival (1). Nowadays we take it for granted that more than 50% of children with ALL can be cured.

In 1956, childhood ALL could therefore have been termed "precurable," rather than incurable. Vincent DeVita, in his textbook, lists sixteen different types of cancer "curable in advanced stages by chemotherapy," another four "curable in the adjuvant setting by chemotherapy," and the remaining 28 tumors as incurable (2). If we extend this concept further, the remaining "incurable" cancers should now be termed "precurable."

Webster's Third International English Dictionary lists only two words having to do with curability: *curable*—capable of being cured, susceptible to remedy; and *incurable*—impossible to cure. There is no entry for *precurable,* a word which Jim Holland started to use in 1983 (1), and which speaks for his unrelenting pursuit of cancer cures. I firmly believe that this word, by virtue of its importance as a concept and as validated by his leukemia research, belongs in the next edition of Webster's dictionary.

In this issue of the *Journal,* symposium participants present theoretical and practical approaches to the cure of cancer in celebration of Jim Holland's contributions. Special thanks are due to Irwin Gerber, Clara Irizarry, Pat Byron Katzoff, and Diana Gulias of the Department of Neoplastic Diseases for helping me organize the symposium. Generous support was given by Mr. Derald H. Ruttenberg, a trustee of The Mount Sinai Hospital, and Mr. and Mrs. Mario Astaldi. Additional support from Critical Care America, the Upjohn Co., Knoll Pharmaceuticals, Adria Laboratories, Sandoz Pharmaceuticals, Bristol Laboratories, Mead Johnson, and Schering is also gratefully acknowledged.

References

1. Holland JF. Breaking the cure barrier. J Clin Oncol 1983; 1:75–89.
2. DeVita VT, Jr. Principles of chemotherapy. In: DeVita VT, Jr, Hellman S, Rosenberg SA, eds. Cancer, principles and practice of oncology, 3rd ed. Philadelphia: JB Lippincott, 1989, 276–300.

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. From the Department of Neoplastic Diseases, Mount Sinai School of Medicine, New York. Address reprint requests to the author, Professor of Neoplastic Diseases, Derald H. Ruttenberg Cancer Center, Box 1129, One Gustave L. Levy Place, New York, NY 10029.

James F. Holland
A Brief Synopsis of His Career

James Frederick Holland was born in 1925 in Morristown, NJ. He received his A.B. in chemistry from Princeton University in 1945 and his M.D. from the Columbia University College of Physicians and Surgeons in 1947. He took his house staff training at the Presbyterian Hospital, served in the U.S. Army, and returned to be chief resident and fellow in medicine at Francis Delafield Hospital. He joined the National Cancer Institute in 1953 as senior investigator and initiated the program there in the combination chemotherapy of childhood leukemia. He left to become Chief of Medicine A and Director of the Cancer Clinical Research Center at Roswell Park Memorial Institute, New York State's cancer research facility in Buffalo. In 1973 he was invited by Mount Sinai Medical Center to become chairman of the newly created Department of Neoplastic Diseases and Director of the Cancer Center. In 1982 he was invested as the first Jane B. and Jack R. Aron Professor of Neoplastic Diseases. In 1988 he became director of the newly dedicated Derald H. Ruttenberg Cancer Center at Mount Sinai. Dr. Holland also serves as the scientific director of the T. J. Martell Foundation for Leukemia, Cancer and AIDS Research.

Throughout these years he has remained an active investigator, clinician, and teacher. His research, conducted with colleagues and students, is expressed in over 500 publications and in a major text, Holland and Frei: *Cancer Medicine.* He is particularly known for his work in experimental therapeutics and for his studies in the acute leukemias, osteosarcoma, and breast cancer. He has participated in changing the status of many cancers from "precurable" to curable.

His honors include Phi Beta Kappa, Alpha Omega Alpha, Sigma Xi, the Albert Lasker Award, the David Karnofsky Lectureship of the American Society of Clinical Oncology, the Jeffrey Gottlieb Award from the M.D. Anderson Hospital and Tumor Institute, the American Cancer Society National Annual Award, the Columbia University College of Physicians and Surgeons Gold Medal for Distinguished Achievements in Medicine, the Jacobi Medallion of the Mount Sinai Medical Center, the Katherine Berkin Judd Award from Memorial Sloan Kettering Cancer Center, the first Return of the Child Award from the Leukemia Society of America, and the first Milken Family Foundation Distinguished Clinician Award.

He has been elected president of the American Association for Cancer Research and the American Society of Clinical Oncology and chairman of the Cancer and Leukemia Group B. He served as chairman of the International Trophoblastic Neoplasia Study Group, as the first U.S.–U.S.S.R. Liaison Officer in Cancer Research, and founded and serves as scientific advisor to AORTIC, the African Organization for Research and Training in Cancer.

He is married to Dr. Jimmie C. Holland, professor of psychiatry at Cornell University College of Medicine and Chief of the Psychiatry Service at Memorial Sloan Kettering Cancer Center, and is the father of six children: Diane, Steven, Mary, Sally, Peter, and David.

About James F. Holland

Joseph H. Burchenal, M.D.

I FIRST MET James Holland when he was working with Al Gellhorn at the Francis Delafield Hospital. Because of the friendship of Gellhorn and David Karnofsky, we saw a lot of each other on our services. I don't quite know how to describe Jim in those days. I fall back sometimes on a few things I learned from my wife, who is an amateur ornithologist. Lots of times I've looked at a bird, and it didn't have the right color. She would say: "That's not the regular one, it's a male but it's a juvenile, it doesn't have the bright coloring or the same call." I think maybe that would describe Jim then. He was a great person, but he hadn't developed the breast coloring that he has now, and also his call was not the same. Of a great warrior in the Trojan War, Stentor, it was said that his voice was the equivalent of fifty other men's. Perhaps those characteristics of Jim developed later on.

In 1954 and 1955, Jim and I saw a great deal of each other because we were worried that the remissions in acute leukemia were still not well defined, and that the term was loosely applied. So Jim, Tom (Emil Frei III), and I together wrote the critical definition of a remission in acute leukemia. Later on, Gordon Zubrod at the National Institutes of Health got together the Cancer Chemotherapy National Service Center, and Jim and I worked on it. Out of that center came the Acute Leukemia Task Forces A and B. Jim headed Task Force B, which continued as the Cancer and Leukemia Group B and produced such wonderful results.

In 1964 and 1965 the chemotherapy panel of the International Union Against Cancer gave Jim and me rein to plan a couple of conferences (1–3). At that time we thought that the most exciting tumors were Burkitt's tumor and choriocarcinoma, both geographically somewhat limited, but apparently occasionally curable. At one of these conferences, in Kampala in 1966, Jim spoke on the importance of high-dose chemotherapy for both Burkitt's tumor and acute leukemia. Later Jim and I were out with Jean Bernard in a Volkswagen minibus, sitting rather exposed on the top of it, and sighted a bull elephant in the distance. Jim bugled to the elephant, who immediately put up his ears and started after us. So Jim speaks Elephantese as well as English.

The next year Jim headed a conference on choriocarcinoma in Baguio in the Philippines. The site was picked because choriocarcinoma was much more common in the East than in the West. On one of our sightseeing trips, we went out to a village where all of the families were busy carving wood, making among other things some nice salad bowls. Jim brought home two whole crates full of these bowls. He never does things halfway.

In 1972, together with Engracio Cortes, Jim was the first to show that doxorubicin has an effect on metastatic osteosarcoma (4). In 1974, he started using doxorubicin as adjuvant therapy of osteogenic sarcoma (5). Jim is a strong proponent of randomized trials, but when it was close to proven that a high percentage of cures can be produced in osteogenic sarcoma with adjuvant therapy, he was just as strongly against randomization, arguing that

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. Address reprint requests to the author, Director of Clinical Research Emeritus, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

physicians could not have told thinking parents the honest truth about the benefits of chemotherapy versus no chemotherapy (6). He was certainly stentorian on that stand, and roundly criticized those who persisted in favoring randomization under those circumstances.

In essence, Jim is a distinguished creative scientist and a careful and exact investigator. In this issue, we have a chance to read all sorts of other things about him.

References

1. Holland JF, Hreshchyshyn MM. Chemotherapy of trophoblastic neoplasia. UICC Bull, vol. 4 (No. 3), 1966;
2. Choriocarcinoma. Holland JF, Hreshchyshyn MM, eds. UICC Monograph Series, vol. 3. Heidelberg: Springer-Verlag 1967.
3. Treatment of Burkitt's tumor. Burchenal JH, Burkitt DP, eds. UICC Monograph Series, vol. 8. Heidelberg: Springer-Verlag 1967.
4. Cortes EP, Holland JF, Wang JJ, Sinks LF. Doxorubicin in disseminated osteosarcoma. J Am Med Assoc 1972; 221:1132–1138.
5. Cortes EP, Holland JF, Wang JJ, et al. Amputation and adriamycin in primary osteosarcoma. N Engl J Med 1974; 291:998–1000.
6. Holland JF. Adjuvant chemotherapy of osteosarcoma: no runs, no hits, two men left on base. J Clin Oncol 1987; 5:4–6.

Reminiscences

Dear Jim: What a delight to be included among your colleagues invited to celebrate your birthday. From the beginning, you have played a major role in the development of cancer chemotherapy. As in all beginnings, cancer chemotherapy was started by just a few talented and dedicated people with the imagination and audacity to believe that they might succeed in curing human cancer with chemicals. And succeed they did, at least in about a dozen types of cancer, through a combination of hard work, creativity, and perseverance. Their persistence is especially commendable in the face of many early failures and sometimes not a little derision from their less imaginative colleagues. Some of the early pioneers have died. Such names as Dusty Rhodes, Dave Karnofsky, and Frank Schabel quickly come to mind. These men were, of course, all friends and colleagues of yours, and I could name many more. Others among the original chemotherapists are very much alive, and several of them who were particularly close to you were at the symposium and are published here. The early investigators share many interests and bonds, memories of many failures and disappointments as well as small stepwise successes and conceptual advances, and even an occasional triumph. Along the way, many fruitful collaborations and lifelong friendships were forged. Jim, you have had a central role in the whole grand enterprise. Most of all what I'd like to stress is the common bond of friendship and admiration which all of us have for you. We admire your good humor and integrity, your boundless energy and unflagging optimism, and your unrelenting zeal in pursuing our common goal. You are an inspiration to all of us in the cancer field. Not only your colleagues, but the millions of cancer patients throughout the world owe you a great deal of gratitude for all you have done.

Bayard D. Clarkson, M.D.
Prof. of Medicine, Cornell Univ. Medical College
Chairman, Therapeutic Research Laboratory of Hematopoietic Cell Kinetics
Memorial Sloan-Kettering Cancer Center
New York, NY

It is a great pleasure to honor Jim for being one of the founders of the modern field of cancer therapy and a current leader in many aspects of cancer research and patient care. Moreover, his unbounded enthusiasm and optimism are a true inspiration to all of us involved in the difficult problem of cancer, whether in the care of patients at the bedside, or the unraveling of the malignant behavior of tumor cells at the molecular and cellular levels. I have a particular affinity to Jim because we share early imprinting at the Francis Delafield Hospital of Columbia University under the tutelage of Dr. Gellhorn.

I. Bernard Weinstein, M.D.
Frode Jensen Prof. of Medicine
Prof. of Public Health and Genetics
Columbia Univ. College of Physicians and Surgeons
New York, NY

In a phone call recently, Jim Holland told me his age. Two words came to my mind: "Already," and "Only." "Already," because I remember his physical and intellectual strengths, his physical strength driving him from America to Australia, to Africa, to Europe, his intellectual strength with his constantly renewed imagination opening new fields of knowledge. "Only," if I evoke the high quality and importance of his scientific works, of his actions, in part responsible for the remarkable evolution of medicine and biology today.

"Peaceful water of lakes, moving water of rivers, favor meeting in friendship." I should like to evoke two such "liquid" meetings, which led me to discover the two alliances defining Jim Holland. The first meeting took place on the banks of Lake Victoria in eastern Africa in Uganda, close to crocodiles and hippopotami. We belonged, Jim Holland and I, to one of the first scientific groups invited by Dennis Burkitt to organize the progress of his work, and Jim Holland presented a carefully accomplished clinical study involving a strong knowledge of molecular biology, precise analyses of chromosomes, genes, and viruses on the one hand, and observation in the field of ways of life, environmental factors, on the other hand. The second alliance I discovered when we met in Paris on a bank of the Seine: The alliance of science and culture, of biology and an interest in literature, poetry, and arts. Age is not a limit but an opening, as a great French poet, my friend St. John Perse, said in a translation by an American poet, T. S. Elliott, also a Nobel laureate:

> Age beholds a coolness of evening on the heads
> Breaths of the open sea on every threshold
> And on foreheads
> Both for the wider space.

Professor Jean Bernard
Director Emeritus
Research Institute for Leukemia and
Diseases of the Blood, University of Paris

For me it's an extraordinary privilege to participate in this tribute because Dr. Holland has had a profound effect on my own career. It's indeed an honor to be included. At the symposium, listening to the distinguished speakers, I wondered to myself what I did to earn a place on the podium among them. Joshua Lederberg reminded me that I entered medical school when DNA was discovered. Listening to Professor Jean Bernard reminded me that I graduated from medical school about the time that methotrexate, the first treatment for cancer, was discovered. These were all opportunities to participate in enormous scientific events.

When I completed my graduate training in hematology in 1955, I found myself in the corridors of the National Cancer Institute, the Clinical Center, and on the brink of oncology as a discipline. When Dr. Zubrod, who hired me, and Tom (Emil) Frei, who'd been hired shortly before me, looked at me, fresh out of hematology training, they said, "It might be a good thing to work on leukemia." I wondered why they made that decision until I discovered that Jim Holland, who had recently departed from those hallowed halls to go upward to Buffalo, had left behind some patients in the Clinical Center with acute leukemia. They were children. So I suddenly found myself a pediatrician; evidently Dr. Zubrod and Tom hadn't been able to recruit any pediatricians to work on childhood leukemia. Since Dr. Farber had made the startling observation that temporary palliation of the disease was possible, it seemed the logical thing for me to do. And I've been pursuing Dr. Holland's lead for 35 years. My wife still says to me, "If you're so smart and get all those prizes, why haven't you cured leukemia?"

But things have changed, as Professor Bernard indicates. During my early years at the Cancer Institute, we learned in fact that there were several cancers that were curable—not only choriocarcinoma, but childhood leukemia as well. When I went to Houston, we were on the brink of another revolution, because pediatricians had by then recognized that there was research to be done in cancer. So I was of course immediately recognized as a fraudulent pediatrician. In the general hospital I was excluded from pediatrics, and I had to work on adult leukemia, because that was the expertise I had. In 1965, leukemia in the adult was a disease which could not be cured. Thus at M.D. Anderson Hospital, I had the privilege again of working on a new, emerging field.

After 25 years at Anderson Hospital I went to Bethesda in 1990 for a year's sabbatical. I am working with hope on what I think is another emerging problem, the role of the physician-scientist in the new era of medicine that is upon us: the era not of laboratory disciplines, not of fundamental knowledge, but of clinical research. Unlike many of my colleagues, I believe that clinical research and—more specifically—therapeutic research is in fact a basic science, because we have the privilege of investigating enormously complex problems in the arena in which they occur, in the clinic.

Dr. Holland, I certainly hope that we can continue to look to your leadership for many years into the future.

Emil J Freireich, M.D.
Ainsworth Prof. of
Developmental Therapeutics
Director, Adult Leukemia
Research Program
University of Texas, Houston
Special Asst. to the Director
National Cancer Institute, Bethesda, MD

I first met Jim Holland in 1967 at the third annual meeting of the American Society of Clinical Oncology. I remember it well. I gave the first paper from the Baltimore Cancer Research Center, which was a branch of the National Cancer Institute, and after my talk a person unknown to me leapt up from the front row, ran to the back of the room yelling something about wanting to show a slide, dropped a slide in the projector, and fortunately confirmed and extended some of the observations I was reporting. And when that was over, I, with my heart pounding, sat down next to Danny Bergsagel and said, "Who was that man?" He said, "That was Jim Holland. If you're going to play in this game, you'd better get used to it." Not only did I get used to it, I came to love it. I was extremely proud when Jim appointed me chairman of the committee on acute leukemia of the Cancer and Leukemia Group B years ago, and the only reason that I'm still underpaid and still participating in what I hope will be judged as academic medicine is that during difficult times in my own career, Jim was instrumental in keeping my eye straight on the road that I truly wanted to follow. I thank him for that. Cancer research is an exciting field, and it's an exciting time to be an oncologist. I learned to be excited from Jim Holland, and being excited has not yet let me down.

Peter H. Wiernik, M.D.
Guttman Prof. and Chairman, Dept. of
Medical Oncology
Albert Einstein College of Medicine
Bronx, NY

It's a pleasure to say happy birthday to Jim Holland and to honor his many contributions to medicine and especially to cancer therapy. Sometimes looking back is instructive—for instance, this excerpt from a little Metropolitan Life brochure, "Cancer, a Message of Hope," published about the time Jim was born:

> Cancer can be cured and has been cured,
> X-ray and radium treatment have claimed cures,
> Surgery is the key to curing cancer,
> Early examination,
> He who hesitates is lost.
> Remember medicines are useless,
> They may relieve pain,
> They do not destroy cancer,
> The cancer must be destroyed.
> An operation is for certain,
> See your doctor at once.

About that time, Jim Holland was born, not with a silver spoon in his mouth but with a chemotherapy syringe in his hand. We owe to Jim (and many colleagues) the important ability to have changed this message.

Joseph R. Bertino, M.D.
Prof. of Medicine, Cornell Univ. Medical College
Lymphoma Service, Div. of Hematologic Oncology
Memorial Sloan-Kettering Cancer Center
New York, NY

About three weeks before the symposium honoring Jim Holland, he had an opportunity to "roast" me on my retirement from the Cancer and Leukemia Group B. I told him I was going to have the last word. So here goes. We all know that Jim has many marvelous qualities: high intelligence; total commitment to his family, his patients, his friends; outrageous ties; soft voice; and so forth. But he has two additional qualities.

Number one is enthusiasm, and I think we've all seen that. On a survival plot of children with acute lymphoblastic leukemia following the treatment regimens Jim has instituted in the CALGB since 1955 (see Fig. 1, page 414), Jim's comments on early results began with "Titillating," which then became "Fantastic," and—when the survival curves got up to 70% and 80%—"Monumental." Most people, no matter how enthusiastic, would not have gone further. But our hero didn't stop there; Jim continued to design studies that in fact further improved survival.

The second thing Jim is full of is courage. Courage was a necessity in the early days particularly, when everybody was absolutely certain that an investment in the chemotherapy of cancer, and specifically of leukemia, was poor judgment from the research point of view. They were absolutely sure, and "proved" in a variety of ways, that there was no way to cure cancer. Cynicism masquerading as intelligence was everywhere. But in the end, our hero did it. Enthusiasm, courage, and talent, Jim, you have aplenty!

Emil Frei III, M.D.
Prof. of Medicine
Harvard Univ. School of Medicine
Director and Physician-in-Chief
Dana Farber Cancer Institute
Boston, MA

The Interface of Science and Medicine

Joshua Lederberg, Ph.D.

My only contribution to the field of cancer was a paper I wrote 45 years ago, in which I defended the somatic mutation theory (1), a theory that has proven successful. However, I can discuss with alacrity the interface of science and medicine, since I have had occasion to look into this question from time to time, both in a fairly systematic way and also as part of my daily work at the Rockefeller University. I am happy to share some observations about how I think the science–medicine interface has gone, how it may go in the future, and how it can be directed in its proper path in the future.

The SATT Model. My first response was generated in reaction to some propositions made at an earlier stage in the direction of the National Institutes of Health (NIH). I saw a report that tried to provide some justification for the research programs of NIH by the so-called SATT model. The basic notion is that we begin with the development of fundamental knowledge. We need a large basic science substrate from which technological and clinical applications will be devised. The flow is all in one direction, from basic science to medical practice. And clinicians and patients would all bow down in great gratitude to the illuminations provided by the basic scientists. That may not be a bad idea, but not necessarily for the right reasons.

The "Can of Worms" Model. My own observations about what has happened in the history of science and medicine are best portrayed by what I call the "can of worms" model, in that progress is by no means linear, that no particular direction can be described, that there is constant interplay not only of the applications of basic knowledge to medical practice but of natural historical and clinical observation both inspiring and provoking important discoveries. I venture to say that the most revolutionary discoveries have arisen out of observations that did not fit prevailing scientific doctrine, and required a reexamination of the fundamental concepts.

The most outstanding example I can think of is in the foundation of my own field. Oswald Avery was a biochemist who was commissioned in the 1910s to study the biochemistry of pneumonia at the Rockefeller University. He became an expert on the specific soluble polysaccharide which was the characteristic antigen that defined the different serotypic variants of this organism. This was a necessary basis for the development of serodiagnostic reagents and of serum therapy. Then came the bombshell. In 1928, Fred Griffith in London described an experiment which was ignored by most people, since they could not grasp its significance. He showed that an extract of one pneumococcal type could alter the serologic character of the cells of another (2). Avery proceeded to investigate that particular phenomenon from 1928 till 1944, and in effect established that the foundation of genetic influence appears in a molecule which nobody at that time suspected could possibly have those characteristics (3). This molecule is deoxyribonucleic acid.

Everything we do today in molecular genetics and molecular medicine stems from that source. I do not think those experiments could have been performed except in the context of a clinical observation. The motive, the drive, the financing, the resources for an inquiry so far out of the context of general genetics would have been absent. But in the setting of a medically important and necessary investigation, the experiments were possible. The tools were there: back-

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. Address reprint requests to the author at The Rockefeller University, Suite 400, 1230 York Avenue, New York, NY 10021.

ground information, natural and historical information about pneumococcus, and so forth. Probably the most revolutionary discovery in modern biology arose by this "can of worms" model.

Medical-Scientific Progress

Medical-scientific progress over the last century can roughly be divided into three phases.

Microbe Hunting. Modern scientific medicine began with discoveries about infectious diseases that reached their flowering just 110 years ago, with the work of Louis Pasteur and Robert Koch, and the identification of specific bacteria as the etiologic agents of a variety of diseases. There again an important biological discovery was founded, provoked, inspired, and substantiated by clinical observations, whether the discovery was cholera, tuberculosis, or anthrax. Out of that arose one of the two or three major biological revolutions of the 19th century. I don't want to put down Darwin and Mendel as equal competitors, but the recognition of the world of the microbe, the development of experimental tools for the isolation of specific microbiological agents, the concept as we now know that the etiology of each infectious disease can be traced to some specific pathogenic entity, a different species of microorganism, constituted a biological revolution.

From the 1880s until the 1920s and 1930s, 90% of medical progress consisted of the systematic exploitation of that fundamental insight—the "microbe hunting" that Paul de Kruif described so vividly (4). It was a rather clear paradigm, a highly reductionist one. For every disease, find the bug that causes it, isolate it, use it to develop a vaccine, and in due course use that knowledge for the development of chemotherapeutic agents. We had a highly reductionist approach within that particular sphere. One could start with scientific knowledge of the microbe and from that develop important, necessary, and effective modes of management of disease. This has of course reached its culmination, and 90% of the battle has been won. But we must never forget that it is an eternal battle, and we live with constant reminders at the present time that infectious disease is far from conquered.

Constitutional Diseases. Roughly by the time of World War II, we had the beginnings of the antibiotic industry, a fairly well routinized set of procedures for the development of vaccines, and we rather knew what to do about infectious disease. The new horizons were constitutional diseases. The NIH was founded on the ideological groundings of the work on infection, but then faced a much more difficult problem, trying to deal with the complexities of the human host. When questions such as cancer, psychiatric disorders, and heart disease arise, the answer is not so simple as locating a bug and stamping it out. Investigative protocols are more complicated because you cannot conduct experiments with billions of people overnight as you can with microbes without facing ethical, technical, and economic constraints. Intrinsic biology is also very much more difficult. Trying to drive a wedge between a tumor and its host is intrinsically far more difficult than trying to do that between an alien bacterial invader and its host. It has taken a very long time. During that interval, a great deal of medical progress was made, albeit in the empirical mode. It is hard to point to any important drug that was not discovered by serendipity or empirical observation. That applies to the vast majority of chemotherapeutic agents used in cancer. In large screening programs based on limited rationale, one cannot predict which compound is going to work. One learns ex post facto that some things work better than others. One can do meticulous science in terms of calibration and validation of findings, but one cannot confidently operate from any theory which can predict the next step. The latter is what I mean by rationale.

DNA as Key to Therapeutic Advantage. The breakthroughs that led to the third phase started emblematically in 1944 with Avery's discovery. The knowledge of the role of DNA as the central storehouse of the blueprints of the cell was and is the key to how to approach questions of the distinctions within the human organism and its cells which can be used for therapeutic advantage.

The first clinical application of this knowledge goes back just about ten years ago to Y. W. Kan developing diagnostic procedures for the prenatal diagnosis of sickle-cell disease and thalassemia (5). This was the first time that a clinically significant procedure depended on one's knowing the structure of DNA. My criterion is: Could you have done it without knowing about the double helix? Nothing prior to that time did require that specific knowledge and information. The 1980s have seen a veritable explosion of knowledge in this direction and the rebeginnings of rationally based therapy, using drugs designed not with perfect predictability but rather founded on some specific theory of the nature of the interaction of the chemical entity with the targets, the receptors and the cell they are supposed to address.

That kind of reductionism is much in vogue today, and to some degree it is well founded. There are astonishing developments in our knowledge that simply were not available thirty or forty years ago. I used to be quite impatient, as I have been working in this general area since the 1940s. When were the fruits going to come? Where were we going to see specific useful, clinical applications of the kind of knowledge that other molecular biologists and myself were having a great deal of fun developing? And what good would they be?

Intermediary metabolism, with its energy interconversions, mutual interconversions from nutrients into building blocks, has been the subject of extensive investigation during this past half century, and much of its details have been elucidated. There are about four hundred entities of these interconversions, each of which requires a few or a half dozen enzymes. To deal with that scope of intermediary metabolism probably takes about five thousand genes, genes which are reasonably commonly shared among most organisms on earth and whose structures are to some degree actually conserved from one species to another. Not every cell has every one of the steps. Humans are quite deficient compared even to *Escherichia coli* and certainly to green plants. We require some of these materials as nutrients in our environment. Our assumption is that we have in the course of evolution lost some of those synthetic functions that were originally present in the primordial cell. The fact that a scheme of intermediary metabolism exists at all is the triumph of biochemistry, or premolecular biology if you like, with the development of that kind of insight. Of course we had to have this foundation to do other things, such as therapeutic intervention.

The other reductionist paradigm can be expressed in terms of a physicochemical approach to DNA itself. Here we do not have a map quite as coherent as I can give you for intermediary metabolism; we don't even know the size of the human genome to one significant figure. It's more or less 3 billion nucleotides. This is an approximation, and there may be variations from cell to cell. A reductionist project is currently in operation to try to get down every one of them, spending about $3 billion (6). It can't be done at that price today, but with a little extrapolation this human genome project can be completed for about a dollar a base pair. Out of that, it would be hypothetically possible to build about ten million genes, which is roughly the information content of a few copies of the Encyclopedia Britannica.

We now know that only about 1% of that information is coding information. Maybe another 4% or 5% play some role in the regulatory functions. About 90% of the DNA has no discernible function and may in fact not have one from the perspective of the organism; that percentage exists not because it serves any purpose of the organism, but because it serves some purpose of the DNA. Once a piece of DNA has been smuggled into the genome, it is difficult to see any special procedure by which it can be eliminated. DNA will tend to accumulate in any genome up to a certain point, just out of the dynamics of the molecular basis of replication of that DNA within that chromosome, and may not necessarily have any functions. We know today that the vast majority of mutations that occur in DNA are not subject to any great natural selection but have indifferent results, either not causing a change in the corresponding amino acid, or causing a change that for the most part does not make much difference. Particular genes in closely related species have a lot of variation in protein composition but that variation does not seem to alter their functionality. The stringent NeoDarwinians among us, including myself, used to think that everything we saw was a consequence of a specifically oriented natural selection. I think we have had to abandon that position. There could be a lot of drift in that composition.

At any rate, we end up with the coding of about 100,000 proteins that are necessary to make up the entire body, about 5,000 of those being the housekeeping entities. As a crude guess, any given eucaryotic cell is probably expressing about 10,000 proteins, using 10% of the total genome in a given cell. About half of these proteins are the housekeeping proteins common to most cells, and the other half unique to that particular cell. So there is room in the difference between the 10,000 proteins expressed in one cell and 100,000 available for all the diversification of gene expression which is involved in differentiation, adaptation to different circumstances, and so forth.

We have just scratched the surface; that is to say, we are only a couple of percentage points into a simple complete cataloging of the architectural units of the body, the variety of different proteins involved in its structures, the vast repertoire of enzymes, the enormous variety of growth factors and other control factors.

DNA and Coping with Human Diseases. The agenda for the next two decades is exciting. We know the direction of a large part of biomedical research. It is going to be the exploration of this catalog of DNA information. I not only don't quarrel with that, I am excited about the kinds of prospects it can generate.

What I am concerned with is an excessive reductionist approach: "That's all we have to do." Nothing can be further from the truth. If we were given the complete DNA sequence of a particular human being today, it would still not advance our pragmatic knowledge of how to cope with any specific human disease. It might help a couple of people who are already far down the track of locating specific genes to have some dictionary against which they can match their results. But they can still get their results without the benefit of the complete panoply. My fear is that in the great excitement of this new wave of knowledge, we may lose some of the convergence, the feel for the organism, the natural historical context, the excitement and provocations that come from clinical observation which in my view will be necessary not only to further clinically important needs but even to give us the most important revolutionary findings within biology itself.

References

1. Lederberg J. A nutritional concept of cancer. Science 1946; 104:428.
2. Griffith F. The significance of pneumococcal types. J Hyg 1928; 27:113–159.
3. Avery OT, MacLeod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J Exp Med 1944; 79:137–158.
4. De Kruif P. Microbe hunters. New York: Harcourt, Brace & Co., 1926.
5. Kan YW, Dozy AM. Antenatal diagnosis of sickle-cell anaemia by DNA analysis of amniotic fluid cells. Lancet 1978; 2:910.
6. Watson JD. The human genome project: past, present, and future. Science 1990; 248:44–49.

Cancer Cures

Yesterday, Today, and Tomorrow

JEAN BERNARD

MY INTERPRETATION of the remarkable evolution of medicine and biology can be communicated in the story of three doctors put to sleep and then awakened several decades later.

1900–1935. The first doctor is put to sleep by some witchcraft in 1900 and awakens in 1935. Everything around him has changed. Countries and towns have been transformed, with tractors and trucks in the fields, telephones in the houses, motor cars on the road, and planes in the sky. But medicine has not changed. The physician helps the dying, encounters resistant coughs, liquefies expectoration, but never modifies the course of his patients' diseases. He can only observe powerlessly the evolution of septicemia and meningitis, endocrine deficiency, cancer, leukemias.

1935–1947–1990. The second physician, put to sleep in 1935, is taken out of his lethargy in 1990. He does not recognize anything. Acute meningitis, acute tuberculosis, generalized infection, malignant endocarditis, pneumococcal pneumonias are now curable diseases. Pernicious anemia is pernicious no more. Surgeons open hearts and brains. Hematologists give life to new bodies by changing their blood. Psychiatrists, neurochemists correct sleep disorders. Lumps, radiation, microscopes explore sera, tissues, cells, and even molecules.

For a long period the situation was not as good for cancers and leukemias. For many years, everybody accepted the concept of inevitably fatal diseases, not without reason. The first complete remissions in the history of leukemia were achieved in 1947 in Paris by exchange transfusion (1), and in 1948 in Boston by Sidney Farber with aminopterin (2). Marcel Bessis and I presented the report of the first remission to the French National Academy of Medicine. A master hematologist, a famous old man, remained for a long time over the microscope observing the slide. He hesitated, looked again, then said—so strong was the dogma which considered leukemias inevitably fatal—"These cells look like leukemic cells. But since the patient had a remission, these cells could not be leukemic." Now the situation is absolutely different. I pay tribute to Jim Holland, who, by his personal research on the one hand, and his contributions as head of the Acute Leukemia Group B on the other, is largely responsible for this happy change.

During this period we have learned that biological and medical research progress in two ways. The first pathway goes down from basic research to clinical application. Genetic engineering has already been applied to the prevention of hereditary hemoglobinopathies. The second pathway goes up from clinical observation to basic data. A blood transfusion accident inspired the discovery of the HLA system by my co-worker and friend Jean Dausset (3), and a childhood tumor observed by Dennis Burkitt in Uganda pointed out new mechanisms in the etiology of cancer (4).

1990–2020. What will a physician put to sleep in 1990 find in the year 2020, when he or she awakens? What will medicine be like in the 21st century? This medicine will be dominated more and more by the intelligence of molecular biology. Two metamorphoses which have already begun today will be completed.

The first metamorphosis: our existence will

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. Address reprint requests to the author, Director Emeritus, Research Institute for Leukemia and Diseases of the Blood, University of Paris, 82 rue d'Assas (VI^{e}), Paris, France.

no longer depend on biological phenomena defined only by function. There are precise molecules depending on localized, isolated, cloned genes. This knowledge will go so far that it will be possible to produce these molecules in the laboratory or industry at the end of this century. Some may be used in therapy.

The second metamorphosis: the variety and the intensity of cellular communications will be mapped. Each active cell of the human body receives unread information and instructions coming in from other cells and in turn gives out other information. More and more mediators responsible for these communications are being discovered, as are their receptors. For example, many neuropeptides, especially endorphins, act on receptors and belong to a complex neuroimmunoendocrine system.

The chasm between molecular biology and physiology, first seen in the 1980s, will be bridged during the 21st century, as a new physiology goes forward with new strength. After thousands and thousands of years of barbarousness, this 21st-century medicine will be governed by the advances of molecular biology, and will be able to diminish human unhappiness, especially in the field of Jim Holland: cancers, leukemias, a field in which until the end of the 20th century failures remained common. Too often, 1990s medicine was only "destructive." It destroyed diseased tissues by surgery, radiation, chemotherapy. This medicine was sometimes "substitutive," as in organ transplantation, with good results. A philosopher can then evoke a cannibal order, of "substitutive" medicine, but such a cannibal order cannot be eternal. It will disappear with the progress of "corrective" treatments, and of "etiologic" treatments.

As is often the case for cancers, leukemias have been pioneer diseases. As early as 1948, the two treatments which produced complete remissions, exchange transfusion and aminopterin, were "corrective" and "destructive," respectively. "Destructive" treatments remained the sole treatment for 40 years, but as early as 1980, the first "corrective" treatments for leukemia—interferon, low-dose Ara-C, retinoic acid—were seen (5–7). The initial results were poor, uncertain, irregular, but during the first years of the 21st century, important progress will have been made not only for leukemia but for other cancers, and physicians in 2020 will smile when they recollect the imprecise "destructive" treatments used by their predecessors. They will use new molecules acting on oncogenes, antioncogenes, and they will be able to cure a high proportion of cancers.

An important movement of contemporary medicine is devoted to research into the causes of cancers. This research needs space and time. Inspired by these "etiologic" researches, the medicine of the future will be "preventive" and "predictive." "Preventive," with a better knowledge of disease etiologies, with the progress of vaccination, initially limited to chickenpox, Marek disease, and hepatitis B. Vaccinations preventive of cancers and leukemia will be successfully organized during the first years of the 21st century as a consequence of better knowledge of retroviruses. "Predictive" medicine began around 1970, with the first discovery of the relationship between HLA groups and diseases, for example diabetes, rheumatism, and later, cancer of the pharynx. These will see full expansion during the 21st century. Relationships between genetic factors and environment will be precisely known, analyzed with the means and methods of molecular biology. So "prediction" and "prevention" are closely linked.

The center of the medicine of the future is both "universal" and "individual." For a long time, for too long a time, there has been a great difference between the medicine of rich countries and the medicine of poor countries. In the year 2020: no more difference, for two reasons. First, important information obtained in Uganda and eastern China has brought better understanding of Western cancers. Second, the discovery at the beginning of the 21st century of effective vaccines against malaria and bilharziosis will have diminished the unhappiness of the so-called developing countries. In another sense, "universal" medicine will never be collective, and will remain intensely "individual." We may know many blood groups and tissue group combinations, but each person is unique, different from all others.

Some years ago, following a lecture I gave on the progress of biology to an assembly of cardinals, bishops, religious leaders, in Rome, a famous old cardinal said, "You explain to us, dear doctor, that each man is unique, but we have known this fact for nearly two thousand years." I answered, with respect, that it might be useful to bring biological proofs.

During the last forty years of the 20th century, the marvelous progress of medicine has given birth to a new science, a new discipline, bioethics. Hippocratic morality is no longer sufficient. Humans will have acquired three kinds of mastery: mastery over procreation, mastery over genetics, and mastery over the nervous system. New problems will still confront us, new progress in science, new human duties. But these fundamental principles must be emphasized: respect of human beings, respect of science, denial of self, the responsibilities of researchers, and a necessary humility. Bioethics is the history and geography of medicine in the past, present, and future.

References

1. Bessis M, Bernard J. Remarquables resultats du traitement d'un cas de leuceme aigue par l'exsanguineotransfusion. Bull Mem Soc Med Hop Paris 1947; 63: 871–875.
2. Farber S, Diamond LK, Mercer RD, Sylvester RF Jr, Wolff JA. Temporary remissions in acute leukaemia in children produced by folic acid antagonist, 4-aminopteroylglutamic acid (aminopterin). New Engl J Med 1948; 238:787.
3. Dausset J. Leuco-agglutinins: IV. Leuco-agglutinins and blood transfusion. Vox Sang 1954; 4:190–198.
4. Burkitt DP. Etiology of Burkitt's lymphoma: an alternative hypothesis to a vectored virus. J Natl Cancer Inst 1969; 42:19.
5. Queseda JR, Reuben J, Manning JT, et al. Alpha interferon for induction of remission in hairy cell leukemia. New Engl J Med 1984; 310:15.
6. Baccarani M, Tura S. Differentiation of myeloid leukaemic cells: new possibilities for therapy. Br J Haematol 1979; 42:485–490.
7. Breitman TR, Scolnick SL, Collins SJ. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. Proc Natl Acad Sci 1980; 77:2936–2940.

Protein Kinase C:

Implications for Cancer Prevention and Treatment

I. Bernard Weinstein, M.D.

At the Columbia University College of Physicians and Surgeons we are proud of the fact that Dr. James Holland began his career in oncology at our medical center. Thumbing through reports of the Institute of Cancer Research of Columbia University, I came across a 1951–52 report noting that Dr. Alfred Gellhorn, together with an oncology fellow named Dr. James Holland, carried out a clinical evaluation of a new therapeutic agent supplied by Dr. Alexander Haddow and his colleagues at the Chester Beatty Institute in England. These were truly the beginnings of the modern era of cancer chemotherapy. The effect of this drug on a variety of neoplastic diseases was investigated and it was found to be useful in chronic myeloid leukemia. The next short entry is also of historic significance. The efficacy on thrombocytopenia of a splenic extract, thrombocytosin, obtained from Johnson & Johnson was assessed. No therapeutic value could be determined, but the inspiration for this approach was of course profound; now, colony stimulating factors and lymphokines are commonplace. Several subsequent annual reports described other pioneering studies in which James Holland participated, including early studies on the treatment of hypercalcemia of cancer and a search for a better androgen in the treatment of breast cancer (1, 2).

Molecular Mechanisms Of Carcinogenesis

In my tribute to Jim Holland I want to provide a brief overview of current concepts of molecular mechanisms of carcinogenesis, emphasizing their relevance to cancer prevention and to the development of novel strategies for cancer treatment. I continue a theme Joshua Lederberg introduces in his article: that we are now in a third phase of biomedical research in which fundamental information at the cellular and molecular levels will have a powerful influence on our approach to the cancer problem.

Studies from a number of disciplines have led to an amazingly unifying concept in terms of our understanding of growth control and its disorders in cancer cells. Indeed, the multistage carcinogenic process can be conceptualized as a progressive disorder in signal transduction (3). The cell is controlled by a variety of external growth factors, often polypeptides interacting with membrane-associated receptors, thus leading to the activation of various protein kinases. The phosphorylated proteins then signal events in the nucleus to alter gene expression, either to result in differentiation or to stimulate cell proliferation. The exciting, unifying principle is that a number of the genes mutated in cancer cells, termed "oncogenes," encode proteins that are the stepping stones along these pathways of signal transduction, and that mutations in these genes disturb the related signals. Moreover, carcinogens act by mutating genes that normally control signal transduction or, as in the case of tumor promoters, they act as fraudulent second messengers. This conceptual framework provides a basis for detecting potential environmental carcinogens

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. From the Department of Medicine, Columbia University College of Physicians and Surgeons, New York. Address reprint requests to the author, Director, Comprehensive Cancer Center at Columbia University, Room 1509, 701 W. 168th Street, New York, NY 10032.

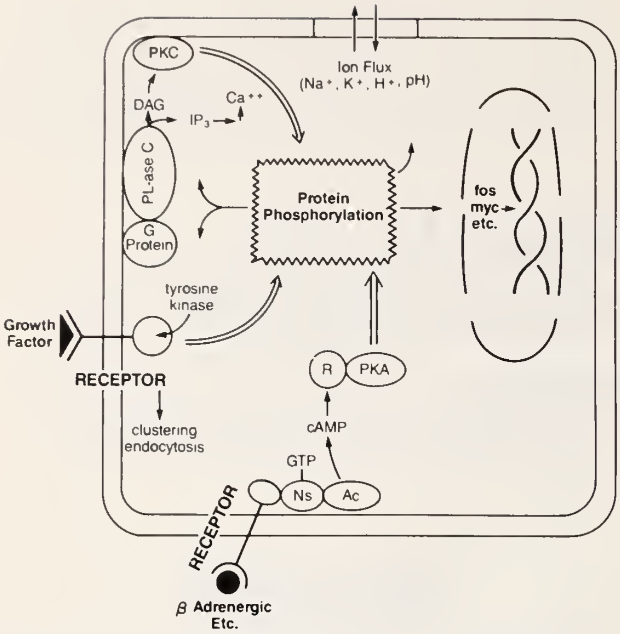


Fig. Schematic diagram of a cell showing various pathways of membrane-associated receptors and signal transduction. Growth factor pathway applies to EGF, PDGF, and insulin. Beta-adrenergic pathway involves coupling via a G regulatory protein (Ns) to adenyl cyclase (AC), and cyclic (AMP) (cAMP) binding to the regulatory subunit (R) of protein kinase A (PKA). Various agonists can activate phospholipase C (PL-ase C), presumably via a G protein, leading to release of diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). DAG activates protein kinase (PKC) and IP_3 causes release of Ca^{2+} from endoplasmic reticulum. These events lead to a cascade of protein phosphorylation that alters the functions of membrane associated receptors, ion channels, and cytoplasmic proteins. Signals (undefined) also enter the nucleus to induce the expression of various genes, including c-*fos* and c-*myc*. Normal cellular oncogenes encode proteins that function at various steps in these pathways. Therefore, mutations in these genes distort the related functions. For example, the *sis* oncogene is a mutant form of the growth factor PDGF, the ErbB oncogene is a mutant form of the EGF receptor, the *mos* oncogene is a mutant form of a cytoplasmic protein kinase, and the *fos* oncogene is a mutant form of a nuclear protein involved in control of gene transcription.

(including dietary factors) and for the design of new therapeutic agents.

The conversion of normal cells to tumor cells is complex and involves a multistep process. In the field of chemical carcinogenesis, we divide this process into three major stages: initiation, which is generally triggered by DNA damage; promotion, which is generally triggered by agents that are not DNA damaging but enhance cell growth and alter gene expression; and progression, the conversion of benign tumors to malignant lesions. The latter stage is the least well understood yet clinically the most important aspect because it is the malignant tumors that present the most difficult challenge with respect to therapy (4).

Tumor Progression. There is emerging evidence that the conversion of benign to malignant tumors is also triggered by agents that cause mutational changes in DNA, and it is in this phase that one often finds gene amplification and chromosomal rearrangements. Once this phase occurs, tumor heterogeneity unfortunately continues to develop. In experimental systems, agents that damage DNA enhance tumor progression, presumably by mutating cellular oncogenes and a second group of genes, the tumor suppressor genes. Deletion in the latter category of genes also perturbs growth. Growth of normal cells is controlled by both positive and negative signals, comparable to an accelerator and a brake in a car. It is not surprising that homeostasis is achieved through both positive and negative signals and that the perturbations that eventuate in cancer cells involve disturbances of both types of function, the oncogenes and tumor suppressor genes, respectively.

Human tumors display a wide variety of oncogene changes resulting from almost every conceivable type of genetic perturbation, including point mutations, translocations, amplification, and deregulated expression. The multistep carcinogenic process that I described in general terms can now be specified in terms of specific genetic changes. This is best illustrated in human colon cancer. From the work of Bert Vogelstein and others, we discern that the evolution of normal colonic epithelia to adenomas of increasing degrees of malignancy and eventually to carcinomas is associated with sequential inactivating mutations in tumor supressor genes and activating mutations in the K-*ras* oncogene (5). Similar findings are being revealed in other human tumors. It seems likely that this knowledge will lead to a molecular subclassification of tumor types, and the rational design of tumor-specific cancer therapy. This knowledge also suggests new strategies for detecting the premalignant stages of cancer, using highly sensitive molecular biology techniques, and new approaches to cancer prevention (6).

In recent years, our laboratory has been interested in mechanisms of tumor promotion and the relevance of promotion to the molecular events mentioned above in carcinogenesis. The compound TPA (12-O-tetradecanoylphorbol-13-acetate) is a potent tumor promotor on mouse skin. It does not damage DNA but it does trigger profound changes in gene expression and stimulates cell proliferation in target cells. Mechanisms underlying these effects are shown in the

Fig. It is now clear that this tumor promotor activates a key enzyme in signal transduction, protein kinase C (PKC), triggering a cascade of events which then leads to the switch-on of genes related to cell division, *fos, myc, jun,* ornithine decarboxylase (ODC), and so on (7). Research in my laboratory has employed derivatives of cells which express extremely high levels of PKC. These cells stably express 10–40 times the normal level of a specific isoform of PKC because they integrate the appropriate cDNA, controlled by a strong promoter/enhancer sequence (8). This produces perturbations in signal transduction pathways that cause the expression of a number of properties of tumor cells, including alterations in morphology and growth control, exaggerated responses to tumor promotors, and decreased dependence on growth factors. These cells are not fully tumorigenic, but they are highly susceptible to malignant transformation by the *ras* oncogene and certain other oncogenes (9).

Similar to the complex scheme depicted by Joshua Lederberg for intermediary metabolism, the signal transduction mechanisms of the cell are also interconnected through a complex network and an almost clockwork-like mechanism. Therefore, a single perturbation in signal transduction is sensed throughout the system. I think this is an important point to bear in mind in terms of the complexity of perturbations that are involved in multistage carcinogenesis. This network is also relevant to therapy since it might be possible to reorient (or reset) the pathways in tumor cells, because effects introduced at various levels in the signal transduction pathway can be perceived by the entire system.

I have termed the multistep conversion of normal cells to tumor cells a progressive disorder in signal transduction, because each step involves further disorders in signal transduction. There are multiple limiting steps. For example, during carcinogenesis it is not sufficient to simply increase the level of PKC, because the same cells may be limited in terms of diacylglycerol, the cofactor that activates that enzyme. Introduction of an activated *ras* oncogene which raises the level of diacylglycerol in cells thus cooperates with the overexpressed PKC. Carcinogenesis is a multistep process because of the necessity to overcome several rate-limiting steps in signal transduction, in order to fully perturb growth control. Fortunately, normal cells are designed so that they are not subject to such overriding perturbations in a single step.

PKC Inhibitors in Therapy. What might be the relevance of PKC for new approaches to therapy? The enzyme appears to be a globular protein. It exists in a folded configuration in the "off" state, and diacylglycerol, phospholipids, and other cofactors activate the enzyme by inducing an open configuration so that substrate proteins can bind to it and be phosphorylated. PKC has a regulatory domain and a catalytic domain, so in theory one could design inhibitors of the enzyme which act on either one of these two domains. Since PKC plays a central role in the action of oncogenes and growth factors, inhibitors of PKC might preferentially inhibit the growth of tumor cells. Several laboratories across the world are now attempting to develop inhibitors of PKC which act either on the regulatory or catalytic domains, or on both domains.

A large number of such inhibitors have been identified (10). A few years ago we discovered that tamoxifen is a fairly potent inhibitor of PKC, acting on its regulatory domain. We suggested the possibility that in addition to its antiestrogen effects, tamoxifen might also inhibit tumors by acting on PKC. There is evidence that tamoxifen's antitumor effect is mediated, at least in part, through a receptor of lower affinity than the estrogen receptor, and therefore we suggested that the former receptor might be PKC. Derivatives of tamoxifen might be developed which have an even greater specificity for PKC. In addition, in the clinical use of tamoxifen one should think about using it and related compounds at doses which are higher than those that target to the estrogen receptor, and in various types of tumors.

Several other inhibitors of PKC look promising. Thus the compound staurosporine is a naturally occurring compound which shows remarkable potency for PKC, but it also inhibits certain other protein kinases. There are, however, recent derivatives of staurosporine that have greater specificity and show some encouraging antitumor effects in transplantable murine models. Since PKC is a multigene family (3, 10) the design of inhibitors that are specific to individual isoforms of PKC might offer additional tumor specificity. Finally, the overall concept of signal transduction and the fact that it is involved in various aspects of tumor formation and growth suggests an entirely new paradigm for cancer therapy.

New Approaches to Prevention as Well as Treatment. Previous approaches to the therapy of cancer have been largely empirical and have generally targeted cellular DNA or biochemical pathways concerned with nucleic acid synthesis or chromosome replication. Thus the agents that have been developed have limited specificity and are often highly toxic to normal replicating tissues. Despite these limitations, the field of cancer chemotherapy developed by James Holland and others has made impressive progress. The recent exciting advances in the study of multistage carcinogenesis, oncogenes, tumor suppressor genes, cell cycle control, growth factors, and signal transduction suggest entirely new approaches to cancer treatment and cancer prevention. Using sophisticated mechanism-based screening assays (that employ recombinant DNA techniques) it should be possible to identify novel natural products and synthetic compounds that inhibit specific steps in signal transduction pathways that play critical roles in the uncontrolled growth of cancer cells. Such inhibitors might act by: 1) Blocking growth factor receptors, 2) Inhibiting specific protein kinases (tyrosine protein kinases, PKC, and others), 3) Altering the function of G proteins, 4) inhibiting specific phospholipases, or 5) Inhibiting specific transcription factors.

Tailoring Therapy to Specific Patients. At the same time, advances in the molecular genetics of specific human tumors should lead to molecular diagnostic approaches so that cancer therapy can be tailored to specific patients, based on the profiles of mutant oncogenes, tumor suppressor genes, and altered signal transduction pathways characteristic of a given tumor. This is essential since the molecular studies on human cancer are revealing that the well-known heterogeneity between tumors (even of the same histology) extends to heterogeneity at the DNA level. For example, only about 40%–50% of malignant human colon tumors have mutations in the c-K-*ras* oncogene. If a drug can be developed that specifically targets the mutant K-*ras* gene, one would want to use it only in those patients that display this mutation, and develop an alternative strategy for colon tumors that display mutations in other critical genes.

Thus, we can look forward to the development of powerful and highly specific new forms of cancer treatment. Elsewhere (10) I have reviewed the relevance of recent advances in molecular oncology to the development of more effective strategies of cancer prevention, including cancer chemoprevention. In summary, thanks to the pioneering studies of Dr. James Holland, major progress has been made in the therapy of cancer. As we look toward the 21st century, recent advances in molecular oncology should open up additional powerful approaches to both cancer treatment and prevention.

References

1. Holland JF, Danielson E, Sahagian-Edwards A. Use of ethylene diamine tetra-acetic acid in hypercalcemic patients. Proc Soc Exp Biol Med 1953; 84:359–364.
2. Gellhorn A, Holland JF, Herrman JB, Moss J, Smelin A. An evaluation of stanolone in treatment of advanced mammary cancer. J Am Med Assoc 1954; 154:1274–1277.
3. Weinstein IB. The role of protein kinase C in growth control and the concept of carcinogenesis as a progressive disorder in signal transduction. Adv Second Messenger Phosphoprotein Res 1990; 24:307.
4. Weinstein IB. The origins of human cancer: molecular mechanisms of carcinogenesis and their implications for cancer prevention and treatment—27th G.H.A. Clowes Memorial Award lecture. Cancer Res 1988; 48: 4135–4143.
5. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61:759.
6. Weinstein IB. Cancer prevention: recent progress and future opportunities. Cancer Res 1991; 51:5080s–5085s.
7. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y. Direct activation of calcium-activated phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J Biol Chem 1982; 257:7847–7851.
8. Housey GM, Johnson MD, Hsiao WLW, O'Brian CA, Murphy JP, Kirshmeier P, Weinstein IB. Overproduction of protein kinase C causes disordered growth control in rat fibroblasts. Cell 1988; 52:343–354.
9. Hsiao WL, Housey GM, Johnson MD, Weinstein IB. Cells that overproduce protein kinase C are more susceptible to transformation by an activated H-ras oncogene. Mol Cell Biol 1989; 9:2641–2647.
10. Weinstein IB. Strategies for inhibiting multistage carcinogenesis based on signal transduction pathways. Mutat Res 1988; 202:413–420.

Enzymes of the Thymidylate Cycle as Targets for Chemotherapeutic Agents:

Mechanisms of Resistance

JOSEPH R. BERTINO, WEI WEI LI, JAMES LIN, TANYA TRIPPETT, ERDEM GOKER, BARRY SCHWEITZER, AND
DEBABRATA BANERJEE

ANTIMETABOLITES continue to be important drugs for the treatment of human malignancies. Their spectrum of antimetabolite activity includes

• Fluoropyrimidines:	Cancers of gastrointestinal tract, breast, bladder, head and neck
• Thiopurines:	Acute leukemia
• Cytosine arabinoside:	Acute leukemia, lymphoma
• Methotrexate:	Acute lymphocytic leukemia (not myeloid); cancers of breast, head and neck; choriocarcinoma; lymphoma

They possess important advantages for cancer chemotherapy, especially with regard to long-term toxicities; they

- Usually are not carcinogenic.
- Usually do not produce sterility.
- Usually are devoid of long-term toxicities (cardiac, pulmonary).
- Involve rapid marrow recovery.
- May be "modulated" to increase effectiveness.

Thus far, clinically useful drugs of this class are inhibitors of nucleic acid synthesis and interfere with cell growth by affecting DNA and/or RNA synthesis. As our knowledge of key processes that cause uncontrolled cell growth increases, inhibitors targeted to these pathways may provide even more useful and selective agents. The definition of an antimetabolite may change in that the drug or chemical (or even an antibody) may compete for the metabolite at the enzyme or receptor site but not resemble it in the classic sense. Knowledge of the mechanism of action of some antimetabolite drugs, as well as knowledge of pathways for their transport, activation, retention, and catabolism, provides important opportunities to understand why tumor cells are intrinsically resistant or acquire resistance to these drugs. Although some antimetabolites, such as cytosine arabinoside, have a narrow spectrum of anticancer activity, others, such as the antifolates and fluoropyrimidines, are used to treat a wide spectrum of human neoplasms. The basis of intrinsic resistance, or sensitivity of human cancers, to these drugs is not well understood, despite the large body of information on intrinsic resistance in experimental tumors.

Mechanism of Methotrexate Action. A fairly complete understanding of methotrexate (MTX) action is now at hand, but we are continually learning more about this drug and folate metabolism. For example, MTX enters most cells by an active carrier transport mechanism used by reduced folates (1–3). Once inside the cell, MTX is polyglutamated, that is, additional glutamates (up to 5) are added. MTX and MTX polyglutamates bind tightly to dihydrofolate reductase, thus inhibiting tetrahydrofolate formation re-

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. From the Program of Molecular Biology and Therapeutics, Sloan-Kettering Institute for Cancer Research, Department of Medicine, Memorial Hospital, New York. Supported by USPHS Grants CA 08010 and CA 47997 and American Cancer Society Grant ACS BC 561C. Address reprint requests to the author, who is American Cancer Society Professor of Medicine, Cornell University Medical College, at Box 78, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

quired for thymidylate and purine synthesis. Dihydrofolate polyglutamates that increase after MTX treatment as well as MTX polyglutamates also inhibit purine biosynthesis (4).

Recently, a second transport pathway for folates and MTX that differs from the reduced carrier pathway has been described (reviewed in 5). Folic acid and 5methyltetrahydrofolate have a greater affinity for this system than MTX (6). This is an endocytic-type pathway in which the drug enters cells by a process which has been called potocytosis (7). The gene for the folate receptor protein has been cloned (8–11), and antibodies to this protein have allowed a study of the distribution of this binding protein to be made in normal and malignant tissue (12). The distribution of this protein is limited to a few normal tissues that include the kidney tubules and the chorioid plexus. Certain tumors, especially ovarian cancer, have high levels of this protein. Thus, these tumors may be a target for monoclonal antibodies or antifolates that enter cells by this transport mechanism (13, 14).

The understanding of the mechanisms of MTX action has also allowed elucidation of various mechanisms by which cells acquire resistance to MTX. The four known mechanisms of MTX resistance are a decrease in the uptake of the drug, a decrease in retention due to defective polyglutamylation, an increase in the enzyme activity, or a decrease in binding of MTX to dihydrofolate reductase. The genetic basis for certain of these mechanisms has been elucidated in MTX-resistant cell lines, particularly gene amplification, and point mutations resulting in amino-acid changes in dihydrofolate reductase (15). The genetic basis for decreased transport or defective polyglutamylation (which could be synthesis or degradation) has not been elucidated as yet. Cloning of the folylpolyglutamate synthetase gene and cloning of the reduced folate transport gene should allow identification of the molecular basis for these resistant phenotypes.

Antimetabolite Resistance in Human Leukemia. Our initial efforts to understand natural and acquired resistance to antimetabolites focused on human leukemia and the antifolate methotrexate (MTX). Acute lymphocytic leukemia (ALL) blasts are inherently sensitive to this drug, whereas the nonlymphocytic types (acute nonlymphocytic leukemia [ANLL]) are usually resistant to it. Thus, it is a key drug in curative regimens for ALL but is not usually employed in treatment programs for ANLL. Acute leukemia blasts may be obtained repeatedly from blood or bone marrow, sometimes in large quantities, and blast types may be identified and quantitated by labeling with appropriate fluorescent antibodies.

We have used a screening assay for MTX sensitivity based on tritium displacement of 5[3H] deoxyuridine as thymidylate is synthesized in suspensions of whole cells (16, 17). This assay allows an assessment of the level of inhibition by various concentrations of MTX and analogs (1, 2). We use a 3h incubation with the drug to approximate the duration of exposure of cells in vivo to pulse doses of MTX (t~ = 3h). To test retention of drug (usually due to polyglutamate formation), we also measure the rate of thymidylate synthesis that persists after the cells are washed free of drug and incubated in a drug-free media for 4h. Our initial studies using this screening assay indicated that ANLL blasts and ALL blasts are equally sensitive to short-term exposure to equimolar concentrations of MTX; in contrast, ANLL blasts recover from this inhibition after incubation in drug-free media, whereas ALL blasts do not (18, 19). In addition, ANLL blasts increase their level of dihydrofolate reductase after exposure to MTX to levels severalfold higher than pretreatment levels when measured 24h after drug exposure (20). The basis for the lack of retention noted in ANLL blasts appears to be impaired polyglutamylation. Levels of long chain (Glu3-Glus) polyglutamates in ANLL cells are lower than in ALL cells after a 24h incubation with [3H] MTX. However, there is considerable variability, suggesting that some patients with ANLL might benefit from pulse MTX (19). The percentage of adults with ALL cured with MTX-containing regimens is less than among children with this disease (about 30% vs. 60%). When we tested ALL blasts from adults, there was less long-chain polyglutamate formation than in blasts from children. There was marked heterogeneity, suggesting that some of these patients might benefit from treatment with MTX, while others might not.

Of interest is that we have found that MTX resistance due to impaired polyglutamylation is schedule dependent (20, 21). As the duration of exposure is increased, the need for polyglutamylation (and retention) for cytotoxicity lessens. However, exposure to relatively high levels of MTX for greater than 48h without leucovorin "rescue" also leads to greater host toxicity; therefore, selectivity may decrease.

Acquired Drug Resistance in Acute Lymphocytic Leukemia. Although many patients with ALL are cured by combination chemotherapy programs that contain MTX, approximately one-third of ALL patients relapse and are believed to be clinically resistant to MTX, as well as to some of the other drugs with which they are treated. The in situ screening assay has also been of help in testing for acquired resistance, although the development of sensitive specific assays that measure dihydrofolate reductase activity levels, binding by MTX, and uptake and polyglutamylation of MTX have made the screening test less essential. As has been found in experimental systems, gene amplification leading to increased levels of DHFR, decreased uptake due to decreased influx, or decreased polyglutamate formation have been found in some patients with acute lymphocytic leukemia clinically resistant to this drug (22–24). Thus far we have not found mutations in the dihydrofolate reductase gene in MTX-resistant leukemia blasts.

Based on these assays it may now be possible to predict which patients might benefit from MTX treatment. The assays employed, especially the in situ assay, also allow the evaluation of new folate analogs and should be useful for guiding new drug development.

Intrinsic Methotrexate Resistance in a Solid Tumor: Soft-Tissue Sarcoma. Initial efforts to use fresh human sarcoma cells for evaluating antifolate resistance with an in situ thymidylate synthase assay using 5[3H] deoxyuridine were unsuccessful due to low thymidylate synthase activity in enzymically disaggregated tumors (25). By incubating tumor cell suspensions in RPMI-1640 medium supplemented with 10% FBS for 3 days, activity of the in situ thymidylate synthesis assay markedly increased, allowing 75% of the samples to be evaluated for antifolate sensitivity (25). By criteria established with CEM lymphoblasts sensitive and resistant to MTX, this assay indicated that most sarcomas (80%) are naturally resistant to MTX. Natural resistance to new folate analogs in clinical trial, 10-ethyl-10-deazaaminopterin (10-EDAM), and trimetrexate was also observed in 60% of the samples. Generally, these results were confirmed by specific tests for resistance mechanisms (25). Resistance mechanisms detected were impaired polyglutamylation of MTX and low-level amplification of the dihydrofolate reductase gene. This latter finding was surprising in view of the fact that these patients had not been previously treated with MTX or other antifolates. We are currently attempting to see if this increase in gene copynumber is a consequence of hyperdiploidy, especially of chromosome 5, the location of the dihydrofolate reductase gene.

The defect in polyglutamate formation found in these cells is under investigation using both fresh cells and cell lines we have established from fresh tumor samples. We plan to characterize both folylpolyglutamate synthetase, the enzyme that adds glutamates to folates and MTX, and the hydrolase enzyme, which hydrolyses folylpolyglutamates to the monoglutamate form. We are evaluating this assay to predict response to antifolates in individual patients with sarcoma. The assay also provides a method for evaluation of new antifolates for clinical trial. We are extending these studies to cervical carcinoma and head and neck cancer to develop an understanding of the mechanisms of natural and acquired resistance in these diseases. Preliminary data on epidermoid cell lines derived from these tumors indicate that polyglutamate formation is impaired in some of these cell lines (unpublished observations). We are currently planning to extend these studies to fresh human tumor samples.

Treatment of Methotrexate-Resistant Tumors with New Folate Analogs. As mentioned, lack of polyglutamylation of MTX in certain human neoplasms leads to poor retention of this drug, especially when administered as a short-term infusion (up to 24h). Normal renewal tissues (bone marrow, gastrointestinal mucosa) also appear to be incapable of forming long-chain polyglutamates (26–28). Therefore, the basis of selectivity of MTX enjoyed by certain responsive tumors (for example, childhood ALL) is lost. A continuous infusion dose schedule would be more effective, but it is also more toxic because of this lack of differential polyglutamylation.

A more effective approach to the treatment of these pulse schedule resistant neoplasms may be the use of drugs like trimetrexate that do not require polyglutamylation for retention, or possibly the use of MTX analogs such as 10-EDAM that might be a better substrate for folylpolyglutamate synthetase, the enzyme that adds glutamates to MTX or reduced folates (29). In addition, the selectivity of a drug such as trimetrexate might be enhanced if used in combination with folinic acid (leucovorin, LV). Selectivity in this circumstance would depend on the relative ability of normal vs. tumor tissue to retain (polyglutamate) LV (30).

We have also explored the use of a folate-depleting enzyme, carboxypeptidase G1 or G2 (CPG1), which hydrolyses the terminal glutamate from folates, given together with trimetrexate, to increase the potency of this antifolate (31). Again, selectivity would depend on the relative depletion of intracellular folates produced by CPG1 in tumor tissue vs. normal stem cells. Presumably, tumor tissue not able to form folate polyglutamates would be more susceptible to this approach.

Summary and Conclusions. This brief review should serve to indicate that it is possible to assess tumor sensitivity to antifolates using fresh human tissue in short-term culture. The assays described differ from general assays of tumor sensitivity, such as the clonogenic assay or assays that measure 3H-thymidine incorporation as an indicator of cell viability (32–34), in that the effect of a drug on a human specific target (dihydrofolate reductase) is measured. These specific assays also may prove to be extremely useful in the detection of acquired drug resistance and for new analog drug development.

In a general sense, this type of assay may eventually also be useful for guiding and selecting treatment for individual patients with other drugs with known mechanisms of action. Knowledge of the basis of tumor resistance is essential to develop new approaches to treatment, such as the use of other folate analogs that may still be effective, and to devise ways in which to selectively inhibit tumor cell growth using new analogs (trimetrexate) and leucovorin.

References

1. Goldman ID, Lichenstein WS, Oliveiro VT. Cancer mediated transport of the folic acid analog methotrexate by the L1210 leukemia cell. J Biol Chem 1968; 243:5007.
2. Sirotnak FM, Gontas LJ, Mines LS. Extent of requirement for folate transport by L1210 cells for growth and leukemogenesis in vivo. Cancer Res 1985; 45:4732.
3. Henderson GB. Transport of folate compounds into cells. In: Blakeley RL, Whitehead VM, eds. Folates and pterins: nutritional, pharmacological, and physiological aspects, vol. 3. New York: Wiley Interscience 1986; 20.
4. Allegra CJ. Antifolates. In: Chabner BA, Collins JM, eds. Cancer chemotherapy: principles and practice, chap. 5. JB Lippincott, 1990; 110.
5. Antony AC. The biological chemistry of folate reception. Blood 1992; 79:2807–2820.
6. Antony AC, Utley CS, Van Horne KC, Kalhouse JF. Isolation and characterization of a folate receptor from human placenta. J Biol Chem 1981; 256:9684.
7. Anderson RGW, Kamen BA, Rothberg KG, Lacey SW. Potocytosis: sequestration and transport of small molecules by caveolae. Science 1991; 255:410.
8. Sadasivan E, Rothenberg SP. The complete amino acid sequence of a human folate binding protein from KB cells determined from the cDNA. J Biol Chem 1989; 264:5806. Correction: 1990; 265:1821.
9. Lacey SW, Sanders JM, Rothberg KG, Anderson RGW, Kamen BA. Complementary DNA for the folate binding protein correctly predicts anchoring to the membrane by glycosylphosphatidylinositol. J Clin Invest 1989; 84: 715.
10. Elwood PC. Molecular cloning and characterization of the human folate-binding protein cDNA from placenta and malignant tissue culture (KB) cells. J Biol Chem 1989; 264:14893.
11. Ratnam M, Marquardt H, Duhring JL, Freisheim JH. Homologous membrane folate binding proteins in human placenta: cloning and sequence of a cDNA. Biochemistry 1989; 28:8249.
12. Watman SD, Laik RH, Coney LR, Fort DN, Frasca V, Zurawski VR Jr, Kaman BA. Distribution of the folate receptor GP38 in normal and malignant cell lines and tissues. Cancer Res 1992; 52:3396–3401.
13. Campbell IG, Jones TA, Foulkes WD, Trowsdale J. Folatebinding protein is a marker for ovarian cancer. Cancer Res 1991; 51:5329.
14. Coney LR, Tomassetti A, Carayannopoulos L, Frasca V, Kamen BA, Colnaghi MI, Zurawski VR. Cloning of tumor-associated antigen: MOv18 and MOv19 antibodies recognize a folatebinding protein. Cancer Res 1991; 51: 6125.
15. Schweitzer BI, Dicker AP, Bertino JR. Dihydrofolate reductase as a therapeutic target. FASAB J 1990; 4: 2241–2452.
16. Yalowich JC, Kalman TI. Rapid determination of thymidylate synthetase activity and its inhibition in intact L1210 leukemia cells *in vitro*. Biochem Pharmacol 1985; 34:2319–2324.
17. Rodenhuis S, McGuire JJ, Narayanan R, Bertino JR. Development of an assay system for the detection and classification of methotrexate resistance in fresh human leukemic cells. Cancer Res 1986; 46:6513–6519.
18. Rodenhuis S, McGuire J, Sawicki W, Bertino JR. Effects of methotrexate and of the "nonclassical" folate antagonist trimetrexate on human leukemia cells. Leukemia 1987; 1:116–120.
19. Lin JT, Tong WP, Trippett TM, et al. Basis for natural resistance to methotrexate in human acute non-lymphocytic leukemia. Leukemia Res 1991; 15:1191–1196.
20. Bertino JR, Sawicki WL, Cashmore AR, Cadman EC, Skeel RT. Natural resistance to methotrexate in human acute nonlymphocytic leukemia. Cancer Treat Rep 1977; 61:667–673.
21. Pizzorno G, Mini E, Coronello M, et al. Impaired polyglutamylation of methotrexate as a cause of resistance in CCRF-CEM cells after short-term, high-dose treatment with this drug. Cancer Res 1988; 48:2149–2155.
22. Pizzorno G, Chang YM, McGuire JJ, Bertino JR. Inherent resistance of human squamous carcinoma cell lines to methotrexate as a result of decreased polyglutamylation of this drug. Cancer Res 1989; 49:5275–5280.
23. Carman MD, Schornagel JH, Rivest RS, et al. Resistance to methotrexate due to gene amplification in a patient with acute leukemia. J Clin Oncol 1984; 2:16–20.
24. Horns RC, Dower NJ, Schimke RT. Gene amplification in a leukemia patient treated with methotrexate. J Clin Oncol 1984; 2:2–7.
25. Trippett T, Schlemmer S, Elisseyeff Y, et al. Defective transport as a mechanism of acquired resistance to methotrexate in patients with acute lymphocytic leukemia. Blood 1992; 80:1158–1162.
26. Li WW, Lin JT, Tong W, Trippett T, Brennan M, Bertino JR. Mechanism of natural resistance to antifolates in human soft tissue sarcomas. Cancer Res 1992; 52:1434–1438.
27. Fabre I, Fabre G, Goldman ID. Polyglutamylation, an im-

portant element in methotrexate cytotoxicity and selectivity in tumor versus murine granulocytic progenitor cells *in vitro*. Cancer Res 1984; 44:3190–3195.

28. Koizumi S, Civit GA, Fine RL, et al. Formation of methotrexate polyglutamates in purified myeloid precursor cells from normal human bone marrow. J Clin Invest 1985; 75:1008–1011.
29. Fry DW, Anderson LA, Borst M, et al. Analysis of the role of membrane transport and polyglutamylation of methotrexate in gut and Ehrlich tumor *in vivo* as factors in drug sensitivity and selectivity. Cancer Res 1983; 43: 1087–1092.
30. Sirotnak FM, De Graw JI, Schmid FA, et al. New folate analogs of the lO-deaza-aminopterin series: further evidence for markedly increased antitumor efficacy compared with methotrexate in ascetic and solid tumor models. Cancer Chem Ther Pharmacol 1984; 12:26–30.
31. Li WW, Bertino JR. Inability of leucovorin to rescue a naturally resistant methotrexate human sarcoma cell line from trimetrexate toxicity. Proc Amer Assoc Cancer Res 1990; 32:342.
32. Romanini A, Chou TC, Bertino JR. Carboxypeptidase G2 enhances trimetrexate cytotoxicity in CCRF-CEM cell lines sensitive and resistant to methotrexate. Adv Enzyme Reg 1989; 28:323–333.
33. Weisenthal LM, Lippman ME. Clonogenic and nonclonogenic *in vitro* chemosensitivity assays. Cancer Treat Rep 1985; 69:615–632.
34. Hamburger AW. *In vitro* evaluation of chemotherapeutic agents. Cancer Invest 1991; 9(6):683–690.

Impact of Cytogenetics and Molecular Genetics on Leukemia Therapy

EMIL J FREIREICH, M.D.

SHORTLY after I joined the National Cancer Institute in 1955, Nowell and Hungerford described cytogenetic studies of chronic myelogenous leukemia (CML) cells showing a shortening of the long arm of Group G chromosome 21 or 22 (1). This finding, termed the Philadelphia chromosome, has remained in the forefront of the emerging knowledge of cytogenetics and molecular genetics for 30 years. In the 1970s, Janet Rowley recognized that this chromosomal shortening was in fact not a loss of genetic material but rather a reciprocal translocation between chromosomes 9 and 22 (2). Subsequent discoveries of oncogenes led to the finding that the *abl* oncogene was in the genetic sequence that was translocated to the breakpoint cluster region (*bcr*), forming the *bcr-abl* gene, a "neogene" which to date has never been discovered in the normal human genome (3). This we now realize is the long-sought Rosetta Stone for the understanding of cancer. The *bcr-abl* gene describes a whole illness unique to human beings. It occurs spontaneously in nature, not in the library, and uniquely produces a form of leukemia whose clinical counterparts and natural history have been well described and understood.

Specific Aneuploidies and Treatment Response in Chronic Myelogenous Leukemia. From a cytogenetic point of view, the discovery of the *bcr-abl* neogene uncovered a new criterion for treatment interventions. Although Galton and many others working on CML had shown that complete clinical and hematological remissions can be achieved with myleran, these remissions were associated with minimal or no change in bone marrow cytogenetic aneuploidy. Since the genetic information which produced the disease remained unaffected, it was predicted that myleran would only palliate, not cure the disease. Clinically, in fact, that proved to be the case. Patients with persistent Philadelphia chromosome (Ph^1) in bone marrow cells following treatment subsequently relapsed. The new target for treatment became Ph^1 negativity.

What about CML patients who do not have the Philadelphia chromosome? Molecular genetic studies revealed that about half of these patients actually have the *bcr-abl* neogene in their leukemia cells, although they lacked the gross chromosomal translocation. And these patients have the same clinical course and response to treatment as those who have the cytogenetic t(9;22) translocation (4). This again supports the observation that the *bcr-abl* neogene was responsible for the entire illness.

Aneuploidies and Treatment Response in Acute Leukemias. What about acute leukemias? For many years, the number of cytogenetic aneuploidies that occurred in acute leukemias was perplexing. At first look, there was the tendency to call them random, until several scientists observed that many were repetitious cytogenetic aneuploidies, in other words, nonrandom (5, 6). But they did not occur in all patients with acute

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. From the Adult Leukemia Research Program, University of Texas, Houston. Address reprint requests to the author, who is Ainsworth Professor of Developmental Therapeutics at the University of Texas, Special Assistant to the Director, National Cancer Institute, Bethesda, MD, and Director, Adult Leukemia Research Program, at Box 55, 1515 Holcombe Boulevard, Houston, TX 77030.

leukemias. Many patients with clinical acute myeloid leukemia had totally different cytogenetic aneuploidies. A specific neogene for this complex illness like the *bcr-abl* gene for CML could not be identified.

The importance of cytogenetics in acute myeloid leukemia (AML) began for me when one of our fellows, R. Holmes, made a clinical observation that no one in a laboratory could make. She had a patient who had an excess of eosinophils and blasts in the bone marrow. She was inquisitive enough and scientific enough to pursue this, and found that the patient had a rare aneuploidy, an inversion 16 chromosome aneuploidy (7). Inversion 16 is a true pseudoploid aneuploidy. There is no demonstrable DNA loss, only pericentric inversion of chromosome 16 which cannot be recognized without banding cytogenetics. The association of marrow eosinophilia and inversion 16 aneuploidy struck her, and she started to look critically and prospectively at all AML patients with inversion 16 aneuploidy. She found inversion 16 aneuploidy in 6.6% of the patients with AML in our clinic. Internationally the percentage is somewhere between 5% and 7%.

But does inversion 16 aneuploidy in AML have any clinical significance? It has enormous significance. The subset of AML patients with inversion 16 aneuploidy have disease with a unique natural history and biology. For one, these patients respond favorably to chemotherapy (8, 9). Another nonrandom aneuploidy which is associated with favorable chemotherapy response is t(8;21) translocation. Other nonrandom aneuploidies in AML, such as minus 5 and minus 7 aneuploidies, have been found to predict for unfavorable responses to chemotherapy (10). It appears that these aneuploidies are not clinically significant except for the treatment effect. With treatment, these aneuploidies became fundamentally important.

The association of specific aneuploidies and treatment response does, however, have implications for the biology of the disease itself. Before cytogenetics, the most significant factor predicting for response to treatment was age, which was thought to be a host effect. We now recognize that age is a tumor effect. Leukemias which are favorable from a cytogenetic and clinical point of view occur in younger persons. Just as acute lymphocytic leukemia (ALL) occurs predominantly in children, so inversion 16 and t(8;21) aneuploidies occur in young adults, and the minus 5 and minus 7 aneuploidies occur in the elderly. Patients with AML have qualitatively different responses to treatment.

To illustrate this, we have studied 725 consecutive AML patients at the M.D. Anderson Cancer Center. They were all evaluated with banded cytogenetic studies. Of the 725 patients, 37% fell into clinical categories that have clear association with prognosis and response to treatment (11). For this 37% and for the remaining 63%, the frequency of response was similar to that for the patient group as a whole. This indicates that knowledge of cytogenetic characteristics did not change the overall response rate, which is about 60%. When the patients with evaluable cytogenetic studies were analyzed further, it was found that there is a difference in response which is qualitative, and not quantitative, for those in "favorable" and "unfavorable" groups. These results have been reproduced worldwide. So the objective response to treatment has been affected fundamentally, and the so-called homogeneous 60% response rate is made up of subsets of patients who are either highly responsive or unresponsive.

Aneuploidies and Quality of Response. But the big difference in the natural history is in the quality of response. Patients who are aneuploid in the "favorable" category constitute all of the long-term survivors in this subset of patients. Patients who are "unfavorable" have a natural history of duration of response which is qualitatively zero long-term survivors or cures. When we combine the improved response rate with the improved quality of response, we get a plot of time to treatment failure that illustrates the proportion of time following diagnosis during which the patient was free of the signs and symptoms of the disease. This is using cytogenetic criteria alone, and if we convert the area under those curves to bar graphs, it demonstrates qualitative differences that are impressive. For the first two years following the diagnosis of inversion 16 disease, we can predict that 75% of the patient's time will be free of disease, and that the patient will have a cure fraction which is twice that of the average patient. The other side of the coin is that patients who have "unfavorable" cytogenetic types, minus 5, minus 7 aneuploidies, will be free of disease only 15% of their time. For the first group, treatment is virtually perfect, almost specific; for the last group, the treatment is almost essentially ineffective (12).

Even more important is that, unlike CML patients, the vast majority of AML patients with nonrandom chromosome aneuploidies who are treated to clinically complete remission return to the diploid state. But we have recently carefully analyzed the metaphase plates in these patients. We found seven patients with inversion 16 disease in whom a single metaphase was aneuploid to the cytogeneticist's evaluation. That is 1 out of 25. With a single metaphase aneuploid, seven out of seven of the patients studied to date relapsed. The median time from this examination to relapse was 50 weeks, almost a year, with a range that was fairly narrow, from 20 to 80 weeks, indicating that the new criteria for diagnosis included not only conversion to diploid cytogenetic status but also persistence of even a minority of aneuploid chromosomes, which predicts a failure of curative treatment (13).

What about the inverse? We found 13 patients with inversion 16 disease who had a diploid cytogenetic study on at least 25 diploid metaphases. Of these, 11 remained in continuous complete remission, which is the equivalent of a cure. One patient relapsed and one died in remission of a treatment complication. Thus a diploid cytogenetic study by a technique such as flow-studies or Southern blotting that is sensitive only to about 5% of aneuploid chromosomes still provides a criterion which is necessary but not sufficient for establishing curative treatment.

We have also studied all the nonrandom chromosome subsets. Of 20 patients with one or more aneuploid metaphases, all 20 relapsed. Of the 51 who had a diploid cytogenetic examination, 24 remained in remission and 22 relapsed.

Molecular Genetics in Chronic Myelogenous Leukemia. The first evidence for really effective treatments for CML was a report by Clarkson that if chemotherapy is intensified for patients in hematologic remission, cytogenetic remission, complete or partial, can be induced in a minority (14). That was of interest. We pursued that, and confirmed his report. But the median duration of these remissions was only about three months, and virtually every patient had recurrence of Philadelphia positivity, with essentially zero cure rates (15).

Interferon brought a radical change in the treatment of CML. Both clinical as well as cytogenetic remissions could be obtained with interferon, a biological agent, without any obvious cytotoxic effects from the drug (16). And what is important about these cytogenetic remissions is not only that some are complete but that they also tend to be stable. We have patients now who have been Philadelphia-chromosome negative for over five years during persistent interferon treatment. The completeness of response tends to predict the quality and duration of response, which led our molecular genetic colleagues to pursue a test more sensitive than cytogenetic assays—that is, a molecular test for the presence of Philadelphia chromosome. Lee in our group was the first to apply the polymerase chain reaction to the t(14:18) translocation (17). This translocation was susceptible because the breakpoint at which the neogene is created was narrow. Lee showed that in lymphoma biology, the presence of the t(14:18) gene by molecular studies had more prognostic significance than the pathology. He also showed that in the favorable histologies which are characterized by the t(14:18), the vast majority of these patients have persistent molecular genetic evidence of the disease, and this predicted a low fraction of cures, which was also true. Lee subsequently examined the Philadelphia chromosome in a similar manner (18). Although the breakpoint cluster region is variable, which makes it difficult to apply the polymerase chain reaction, Lee was the first to take advantage of the fact that there are only two major splicing patterns and it is therefore possible, by reverse transcription, to make the polymerase chain reaction adaptable to the cDNA. We have now studied approximately 20 patients with CML who are in complete cytogenetic remission as a result of treatment with interferon. All of them have persistent *bcr-abl* gene in the blood in a concentration which can be detected by the polymerase chain reaction. We have now a new therapeutic target in molecular genetics, because using the polymerase chain reaction properly can amplify the unique gene a millionfold. The biological significance of these persistent genes remains for the future physician-scientist to work out. But certainly we have entered a new era.

References

1. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960; 132: 1497.
2. Rowley J. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973; 243: 290.
3. Lee MS, LeMaistre A, Kantarjian HM, Talpaz M, Freireich EJ, Trujillo JM, Stass SA. Detection of two alternative bcr/abl mRNA junctions and minimal residual disease in Philadelphia chromosome positive chronic myelogenous leukemia by polymerase chain reaction. Blood 1989; 73:2176–2170.
4. Shtalrid M, Talpaz M, Blick M, et al. Philadelphia negative chronic myelogenous leukemia with breakpoint cluster region rearrangement: molecular analysis, clinical characteristics, response to therapy. J Clin Oncol 1988; 6:1569.
5. Trujillo JM, Cork A, Ahearn MJ, Youness EL, McCredie KB. Hematologic and cytologic characterization of 8/21 translocation acute granulocytic leukemia. Blood 1979; 53:695.
6. Sandberg AA, Takagi N, Sofuni T, Crosswhite LH. Chro-

mosomes and causation of human cancer and leukemia: V. Karyotypic aspects of acute leukemia. Cancer 1968; 22:1268.

7. Holmes R, Keating MJ, Cork A, et al. A unique pattern of central nervous system leukemia in acute myelomonocytic leukemia associated with inv(16)(p13q22). Blood 1985; 65:1071.
8. Arthur DC, Bloomfield CD. Partial deletion of the long arm of chromosome 16 and bone marrow eosinophilia in acute nonlymphocytic leukemia: a new association. Blood 1983; 61:994.
9. Larson RA, Williams SF, LeBeau M, et al. Acute myelomonocytic leukemia with abnormal eosinophils and inv(16) or t(16:16) has a favorable prognosis. Blood 1986; 68:1242.
10. Keating MJ, Cork A, Broach Y, et al. Toward a clinically relevant cytogenetic classification of acute myelogeneous leukemia. Leuk Res 1987; 11:119.
11. Freireich EJ. The impact of cytogenetics and molecular genetics on diagnosis and treatment. In: Freireich EJ, Marmont AM, McCulloch EA, Rees JKH, Reizenstein P, Wiernik PH, eds. New approaches to the treatment of leukemia. Berlin: Springer-Verlag, 1990; 173–186.
12. Keating MJ, Smith TL, Kantarjian H, et al. Cytogenetic pattern in acute myelogenous leukemia: a major reproducible determinant of outcome. Leukemia 1988; 2:403–412.
13. Freireich EJ, Cork A, Stass, SA, et al. Cytogenetics for detection of minimal residual disease in acute myeloblastic leukemia. Leukemia 1992; 6:500–506.
14. Cunningham I, Gee T, Dowling M, et al. Results in the treatment of Ph^1 + chronic myelogenous leukemia with an intensive treatment regimen (L-5 protocol). Blood 1979; 53:375–395.
15. Kantarjian HM, Vellekoop K, McCredie KB, et al. Intensive combination chemotherapy (ROAP) and splenectomy in the management of chronic myelogenous leukemia, Blood 1983; 62:689.
16. Talpaz M, McCredie K, Mavligit GM, Gutterman JU. Leukocyte interferon-induced myeloid cytoreduction in chronic myelogenous leukemia. Blood 1983; 62:689.
17. Lee MS, Chang KS, Cabanillas F, Freireich EJ, Trujillo JM, Stass SA. Detection of minimal residual cells carrying the t(14;18) by DNA sequence amplification. Science 1987; 237:175.
18. Lee MS, Chang KS, Freireich EJ, Kantarjian HM, Talpaz M, Trujillo JM, Stass SA. Detection of minimal residual bcr/abl transcripts by a modified polymerase chain reaction. Blood 1988; 72:893.

Consistent Genetic Abnormalities in Human Cancers as Targets for Selective Therapies

Bayard D. Clarkson, M.D.

A recent paper by Gianni Bonadonna listed a number of human cancers that are curable, subcurable, and precurable (1). He listed 8 cancers that are curable in the majority of patients, about a dozen that are curable in a minority of patients, and another group that are responsive to chemotherapy but not curable with existing regimens. Unfortunately the number of human cancers that can be cured with some regularity has not changed greatly during the last decade, and Bonadonna's current list of curable tumors is disturbingly similar to that compiled 10 years ago (2, 3). Thus, while I suspect some investigators will disagree, my own impression is that we have more or less reached a plateau in what can be accomplished with currently available drugs. As examples I will first review the current status of treatment of four human tumors with which I am familiar and then discuss possible future approaches to developing more selective therapies. While these four tumors are all highly responsive to many cytotoxic drugs and can be cured in some patients, despite a great deal of effort their cure rates have not improved substantially in recent years.

Current Status of Treatment

High-Grade Lymphoma. High-grade lymphoma is one of the tumors in which more than 50% of patients can be cured (1), but in our own experience, treatment with the newer so-called third-generation protocols have not significantly improved the overall cure rate (4, 5). With extremely high doses of chemotherapy plus irradiation to bulky tumor masses and rescue with autologous bone marrow transplantation (BMT), it is possible to cure the majority of younger patients with the worst prognoses, whereas only about 20% of such patients would be expected to be cured with conventional protocols (6). However, the therapeutic advantage for such superintensive treatment is marginal. If one waits until such poor-prognosis patients relapse from conventional treatment or performs the autotransplant after they fail the intensive induction treatment, the cure rate, even with autologous transplantation, is only about 20%, or about the same as would be achieved by conventional intensive chemotherapy protocols in this high-risk group of patients. Because many patients with high-grade lymphomas are too old or too ill to tolerate extremely intensive therapy and bone-marrow transplantation, this option is only available to a minority of patients.

Acute Lymphoblastic Leukemia. There has also been relatively little improvement in recent years in the treatment of adults with acute lymphoblastic leukemia (ALL). The excellent results that Jim Holland and his colleagues have pioneered in children with ALL are well known, but despite many attempts during the past several

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. From Cornell University Medical College, and Memorial Sloan-Kettering Cancer Center. Address reprint requests to the author at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

decades to similarly improve the results in adults with ALL, the cure rate has unfortunately remained constant, around 20%–30% (7–9). The worse results in adults are partly due to an increased incidence of specific types of ALL which have naturally unfavorable prognoses, such as Philadelphia-chromosome positive ALL, and partly due to the fact that children tolerate aggressive treatment better than adults.

Acute Myelocytic Leukemia. In acute myelocytic leukemia (AML), if there is no patient selection, only a minority of adults are currently being cured, probably less than 15% overall (10), although again the results are better in children. Except for a few subtypes of AML, such as acute promyelocytic leukemia (9, 11), the cure rates for most types of AML have not improved substantially during the last 15 years or so (10, 12). Patients with AML who have a preexistent myelodysplastic syndrome, especially if they have abnormalities of chromosomes 5 and 7, have an even worse prognosis and are almost never cured (10). Intensive chemotherapy with or without total body irradiation followed by allogeneic BMT may cure some patients with AML who could not otherwise be cured. Patients with AML who relapse from their initial chemotherapy almost invariably die despite further chemotherapy, but a small percentage of such patients can be cured by intensive therapy and bone-marrow rescue. However, once again the advantage of highly intensive therapy and BMT is rather marginal because, for a variety of reasons, only a minority of patients actually come to transplantation (10, 13).

Chronic Myelogenous Leukemia. Of the four tumors I've chosen as examples, the least improvement has been made in the treatment of chronic myelogenous leukemia (CML). Except for a small minority of patients who have been cured by aggressive therapy and allogeneic (or syngeneic) BMT, no patients have been cured, and the overall survival has only improved slightly during the last 65 years.

Possible Future Approaches

If it is true that we have reached a plateau in our ability to cure cancers with the drugs we have at hand, is this a reason for despair? I think not. In fact what is remarkable is that any cancers have been cured with the relatively nonselective drugs available. For the reasons I discuss below, I believe we are now on the threshold of a new era in pharmacology which will eventually lead to the development of selective therapies and increase the curability of many types of cancer.

Over 100 years ago European pathologists noted that nuclear and mitotic abnormalities were common in many human cancers, and it was suspected that these abnormalities might have an important role in the pathogenesis of the cancer (14). A systematic somatic mutation theory of cancer was first proposed by Theodor Boveri in 1914 (15). He suggested that chromosomal abnormalities (the word "gene" had not yet been coined) were the primary changes responsible for the causation and evolution of cancers. This was 30 years before the classic experiments of Avery, MacCleod and McCarty in 1944 first demonstrated that DNA was the primary carrier of genetic information, and over 50 years before modern banding methods were developed for examining chromosomes which allowed one to detect specific abnormalities more readily (9). Not until 1960, or nearly 50 years after Boveri's proposal, did Nowell and Hungerford report on the first specific chromosome abnormality in a human cancer, namely the Philadelphia chromosome in chronic myelogenous leukemia (CML) (16). Beginning with the discovery of the Philadelphia (Ph) chromosome, specific chromosome abnormalities have since been described in many other human cancers (9, 17). The abnormalities include translocations, duplications, additions, deletions, insertions, and other structural changes, as well as submicroscopic abnormalities such as gene amplifications and single nucleotide substitutions. Some of the chromosome alterations are primary tumor-specific abnormalities which are presumably critically involved in the initiation of the particular tumors [t(9;22) in CML, or t(15;17) in acute promyelocytic leukemia], while other changes, which are generally less consistent and less specific, are associated with clonal evolution and progression of the tumor to a more malignant phenotype. Probably all spontaneous human cancers will eventually be shown to have one or more specific genetic abnormalities if one includes abnormalities only detectable by molecular methods of analysis.

Many of the tumor-specific chromosomal abnormalities have been shown to be closely associated with involvement of proto-oncogenes or suppressor genes (9). More than one proto-oncogene may be involved as the tumor progresses from a benign tumor to a more malignant cancer. In experimental tumors, two or more "cooperating" oncogenes may be required for full malignant transformation (18), and similar findings are now being recognized in the evolution of human tumors. For example, Vogelstein and his colleagues have described a series of changes that occur during colorectal tumor progression from adenomas to malignant cancers (19). Varmus has noted that many of the proto-oncogenes that are involved in the pathogenesis of human cancers have been highly conserved during evolution and that they have fundamental roles in regulating cell growth and differentiation (20). The mutations affecting proto-oncogenes may be quantitative, for example altering the level of gene expression, or qualitative, as in translocations, deletions, or base substitutions that alter the physical or biochemical properties of the gene product. Some mutations are recessive in that they inactivate genes (or their products) which normally function in suppressing development of tumors (21, 22).

The protein products of most proto-oncogenes appear to function normally at different points in the regulatory pathways in the cytoplasm or at transcriptional control sites in the nucleus to regulate the expression of genes concerned with growth and development (20). The intracellular regulatory pathways are interrelated and highly complex, and the critical targets for interaction with cellular and nuclear oncoproteins are still poorly understood.

Roles of *bcr* and *c-abl*. Two genes, *bcr* and *c-abl*, appear to have roles intimately involved in the pathogenesis of several types of Philadelphia (Ph)-chromosome positive leukemia. In the t(9;22) translocation consistently associated with CML, the *c-abl* oncogene is transposed from its normal position on chromosome 9 (q34) to a 5.8kb breakpoint cluster region (*bcr*) on chromosome 22(q11), forming an abnormal fusion *bcr*/*abl* gene (23). In the chronic phase of CML the breakpoint usually occurs between the second and third or between the third and fourth *bcr* exons, and the fused *bcr*/*abl* gene transcribes a novel 8.5kb mRNA which in turn encodes a p210kD protein which has protein tyrosine kinase activity greater than that of the normal c-*abl* protooncogene (24).

The breakpoints for the related *bcr*/*abl* gene encoding the $p185^{bcr/abl}$ protein commonly found in Ph-positive acute lymphoblastic leukemias are located in a 20kb region at the 3′ end of the first BCR intron (25–27). The $p185^{bcr/abl}$ protein has about fivefold higher tyrosine kinase activity than $p210^{bcr/abl}$ (28), and this correlates with the former's much more frequent association with an acute rather than a chronic form of leukemia (29–31), and with its greater transforming potency in both in vitro (32–34) and animal experimental systems (35–38). The *abl* sequences of the *bcr*/*abl* genes are unchanged except for loss of the first exon, and this loss alone does not endow c-*abl* with the ability to transform cells (39, 40). However, *bcr* first-exon sequences potentiate tyrosine kinase activation and transforming ability when they are fused to the second exon of c-*abl*, presumably by specifically interfering with negative regulation of *abl*-encoded tyrosine kinase (41, 42).

The *bcr* gene has recently been shown to have a novel type of kinase activity which is confined to a segment encoded by the first exon (43). The *bcr* binding domain is localized in the first exon of *bcr*, and *bcr* sequences bind specifically to the SH2 (src homology region 2) regulatory domain of *abl* in a nonphosphotyrosine-dependent manner. The protein fragments appear to fold back on each other to form a second link at the SH2 regions, and this binding appears to be essential for *bcr*-*abl*-mediated transformation (44). The transforming potential of *bcr*/*abl* oncoproteins appears to be related not only to increased tyrosine kinase activity but also to localization to the cell membrane, where the protein may more readily interact with critical membrane-associated substrates such as growth factor receptors.

What functional changes do the *bcr*/*abl* oncoproteins cause in the affected cells? In chronic phase CML, the changes appear to be quite subtle. The leukemic cells retain their ability to mature, but there are a variety of subtle abnormalities which we have referred to as discordant maturation (45–47). The proliferative advantage of the leukemic cells is relatively slight, because there is a long preclinical period between the initial translocation and the leukemic population becoming dominant. The $p210^{bcr/abl}$ protein presumably somehow distorts the normal transmission of messages in the signal transduction pathways, resulting in faulty programming of the genes necessary for the orderly and well-integrated control of proliferation and maturation. The dysregulation of the genetic programming results in asynchronous maturation of the nucleus and the cytoplasm and various subtle dysplastic and biochemical changes. We believe that the affected Ph+ stem cells are relatively unaffected, and that the abnormalities do not become manifest until the maturation process begins. In the maturing progenitor cell compartments, the lag in nuclear maturation enables the leukemic progenitors to undergo one or more additional divisions than do comparable normal progenitors. The discordant maturation is also associated with an increased life span, further contributing to the increase in the myeloid mass. After an average course of several years, additional genetic abnormalities occur as the disease progresses to the blastic phase. Presumably this is associated with further distortion of signals in the regulatory networks, eventually resulting in the cell's inability to mature.

To develop improved treatment in the future, many investigators are now searching for more selective agents to treat CML and other human neoplasms, including such possible agents as specific tyrosine kinase inhibitors, anti-sense molecules, ribozymes, or some type of immunologic therapy (9). Because the signal transduction pathways are highly complex and the specific abnormalities in CML are still so poorly understood, formidable obstacles remain in all of these approaches (47). Nevertheless it is quite possible that a more effective form of treatment will be discovered before the abnormalities are fully understood, as has recently been demonstrated by the development of highly successful treatment for acute promyelocytic leukemia (APL) (9, 11). Formerly the most rapidly lethal form of leukemia, APL may, it now appears, be curable in the majority of patients by combining treatment with a differentiating agent, all-trans retinoic acid, and cytolytic drug therapy. Retinoic acid is the first example of a selective form of treatment for a leukemia related to a specific genetic abnormality, and this may have broad implications for developing selective therapies for other types of leukemia (11).

The problems facing us in developing more selective therapies are difficult and complex and much work remains (9). However, I am optimistic that we will soon begin to see selective forms of treatment for other types of leukemia as well as solid tumors. This will be a natural progression of the cancer chemotherapy efforts started by Jim Holland and a relatively small number of other early pioneers, including some of the contributors to this issue. We must never forget our gratitude to Jim and the other early cancer chemotherapists for starting us on the road to the eventual conquest of cancer.

References

1. Bonadonna G. Special Article: Does chemotherapy fulfill its expectations in cancer treatment? Ann Oncol 1990; 1:11–21.
2. De Vita VT, Henney JE, Hubbard SM. Estimation of the numerical and economic impact of chemotherapy in the treatment of cancer. In: Burchenal JH, Oettgen HF, eds. Cancer: achievements, challenges, and prospects for the 1980s, vol. 2. New York: Grune & Stratton, 1981; 859–880.
3. Clarkson BD. The elusive goal: Presidential Address. Cancer Res 1981; 41:4865–4884.
4. Schneider AM, Straus DJ, Schluger AE, et al. Treatment results with an aggressive chemotherapeutic regimen (MACOP-B) for intermediate- and some high-grade non-Hodgkin lymphomas. J Clin Oncol 1990; 8:94–102.
5. Straus DJ, Wong GY, Liu J, et al. Small non-cleaved cell lymphoma (undifferentiated lymphoma-Burkitt's type) in American adults: results with treatment designed for acute lymphoblastic leukemia. Am J Med 1991; 90:328–337.
6. Gulati SC, Shank B, Black P, et al. Autologous bone marrow transplantation for patients with poor-prognosis lymphoma. J Clin Oncol 1988; 6:1303–1313.
7. Gaynor J, Chapman C, Little C, et al. A cause-specific hazard rate analysis of prognostic factors among 199 adults with acute lymphoblastic leukemia: The Memorial Hospital experience since 1969. J Clin Oncol 1988; 6:1014–1030.
8. Clarkson B, Gaynor J, Little C, et al. Clinical trials in adults with acute lymphoblastic leukemia at Memorial Sloan-Kettering Cancer Center. In: Gale RP, Hoelzer D, eds. Acute lymphoblastic leukemia. New York: Alan R. Liss, 1990; 231–252.
9. Clarkson B. New pharmacologic approaches to treatment of leukemia. In: Gale RP, Simone JD, Hoelzer D, Frei E, eds. Curing leukemia—Proceedings of the Keystone Symposia Workshop held in Martha's Vineyard, Massachusetts, November 26–28, 1990. Sem Hematol 1991; (Suppl 4):99–115.
10. Clarkson B, Berman E, Little C, et al. Update on clinical trials of chemotherapy and bone marrow transplantation in acute myelogenous leukemia in adults at Memorial Sloan-Kettering Cancer Center (MSKCC) 1966 to 1989. In: Gale RP, ed. Acute myelogenous leukemia: progress and controversies. UCLA Symposia on Molecular and Cellular Biology, New Series, vol. 134. New York: Wiley-Liss, 1990; 239–272.
11. Clarkson B. Review: Retinoic acid in acute promyelocytic leukemia: the promise and the paradox. Cancer Cells 1991; 3:211–220.
12. Berman E, Heller G, Santorsa J, et al. Results of a randomized trial comparing Idarubicin and cytosine arabinoside with daunorubicin and cytosine arabinoside in adult patients with newly diagnosed acute myelogenous leukemia. Blood 1991; 77:1666–1674.
13. Berman E, Little C, Gee T, O'Reilly R, Clarkson B. Reasons that patients with acute myelogenous leukemia do not undergo allogeneic bone marrow transplantation. N Engl J Med 1992; 326:156–160.
14. Von Hansemann D. Ueber asymmetrische zelltheilung in epithelkrebsen und deren biologische bedeutung. Virchows Arch [A] 1890; 119:299–326.
15. Boveri T. The origin of malignant tumors. English translation by M. Boveri. Baltimore: Williams & Wilkins, 1929.
16. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960; 132:1497.
17. Heim S, Mitelman F, eds. Cancer Cytogenetics. New York: Liss, 1987.
18. Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 1983; 304:592–602.
19. Fearon ER, Vogelstein BA. A genetic model for colorectal tumorigenesis. Cell 1990; 61:759.
20. Varmus H. An historical overview of oncogenes. In Weinberg RA, ed. Oncogenes and the molecular origins of

cancer. New York: Cold Spring Harbor Laboratory Press, 1989; 3–44.

21. Stanbridge EG, Cavenee WK. Heritable cancer and tumor suppressor genes: a tentative connection. In Weinberg RA, ed. Oncogenes and the molecular origins of cancer. New York: Cold Spring Harbor Laboratory Press, 1989; 281–306.
22. Stratton MR, Moss S, Warren W, et al. Mutation of the p53 gene in human soft tissue sarcomas: association with abnormalities of the RB1 gene. Oncogene 1990; 5:1297–1301.
23. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 1984; 36:93–99.
24. Konopka JB, Witte ON. Detection of *c-abl* tyrosine kinase activity *in vitro* permits direct comparison of normal and altered *abl* gene products. Mol Cell Biol 1985; 5: 3116–3123.
25. Hermens A, Heisterkamp N, von Lindern M, et al. Unique fusion of *bcr* alpha *c-abl* genes in Philadelphia chromosome positive acute lymphoblastic leukemia. Cell 1987; 51:33–40.
26. Chen SJ, Chen Z, Grausz JD, et al. Molecular cloning of a 5′ segment of the genomic *phl* gene defines a new breakpoint cluster region (bcr2) in Philadelphia-positive acute leukemias. Leukemia 1988; 2:634–641.
27. Denny CT, Shah N, Ogden S, et al. Localization of preferential sites of rearrangement within the BCR gene in Philadelphia chromosome positive acute lymphoblastic leukemia. Proc Natl Acad Sci USA 1989; 86:4254–4258.
28. Lugo TG, Pendergast A-M, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of *bcr-abl* oncogene products. Science 1990; 247:1079–1082.
29. Dreazen O, Klisak I, Rassool F, Goldman JM, Sparkes RS, Gale RP. Do oncogenes determine clinical features in chronic myeloid leukaemia? Lancet 1987; 1:1402–1405.
30. Kurzrock R, Gutterman JU, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemias. In Epstein FH, ed. Mechanisms of disease. N Engl J Med 1988; 319:990–998.
31. Clark SS, Christ WM, Witte ON. Molecular pathogenesis of Ph-positive leukemia. Ann Rev Med 1989; 40:113–122.
32. Daley GQ, McLaughlin J, Witte ON, Baltimore D. The CML-specific P210 *bcr/abl* protein, unlike *v-abl*, does not transform NIH-3T3 fibroblasts. Science 1987; 237: 532–535.
33. McLaughlin J, Chianese E, Witte ON. *In vitro* transformation of immature hematopoietic cells by the P210 *BCR/ABL* oncogene product of the Philadelphia chromosome. Proc Natl Acad Sci USA 1987; 84:6558–6562.
34. McLaughlin J, Chianese E, Witte ON. Alternative forms of the BCR/ABL oncogene have quantitatively different potencies for stimulation of immature lymphoid cells. Mol Cell Biol 1989; 9:1866–1874.
35. Daley GQ, Van Etten R, Baltimore D. Induction of chronic myelogenous leukemia in mice by the $P210^{bcr\text{-}abl}$ gene of the Philadelphia chromosome. Science 1990; 247: 824–830.
36. Elefanty AG, Hariharan IK, Cory S. *bcr-abl,* the hallmark of chronic myeloid leukaemia in man, induces multiple haemopoietic neoplasms in mice. EMBO J 1990; 9: 1069–1078.
37. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale P, Groffen J. Acute leukaemia in *bcr/abl* transgenic mice. Nature (London) 1990; 344:251–253.
38. Kelliher MA, McLaughlin J, Witte ON, Rosenberg N. Induction of a chronic myelogenous leukemia-like syndrome in mice with *v-abl* and *bcr/abl*. Proc Natl Acad Sci USA 1990; 87:6649–6653.
39. Fainstein E, Einat M, Gokkel E, Marcelle C, Croce CM, Gale RP, Canaani E. Nucleotide sequence analysis of human *abl* and *bcr-abl* cDNAs. Oncogene 1989; 4:1477–1481.
40. Franz WM, Berger P, Wang JYJ. Deletion of an N-terminal regulatory domain of the *c-abl* tyrosine kinase activates its oncogenic potential. EMBO J 1989; 8:137–147.
41. McWhirter JR, Wang JYJ. Activation of tyrosine kinase and microfilament-binding functions of c-abl by bcr sequences in bcr/abl fusion proteins. Mol Cell Biol 1991; 11:1553–1565.
42. Muller AJ, Young JC, Pendergast A-M, Pondel M, Landau NR, Littman DR, Witte ON. *BCR* first exon sequences specifically activate the *BCR/ABL* tyrosine kinase oncogene of Philadelphia chromosome-positive human leukemias. Mol Cell Biol 1991; 11:1785–1792.
43. Maru Y, Witte ON. The BCR gene encodes a novel serine/threonine kinase activity within a single exon. Cell 1991; 67:459.
44. Pendergast AM, Muller AJ, Havlik MH, Maru Y, Witte ON. BCR sequences essential for transformation by the BCR-ABL oncogene bind to the ABL SH2 regulatory domain in a non-phosphotyrosine-dependent manner. Cell 1991; 66:161–171.
45. Strife A, Lambek C, Wisniewski D, Wachter M, Gulati SC, Clarkson BD. Discordant maturation as the primary biological defect in chronic myelogenous leukemia. Cancer Res 1988; 48:1035–1041.
46. Strife A, Clarkson B. Biology of chronic myelogenous leukemia: is discordant maturation the primary defect? Sem Hematol 1988; 25:1–19.
47. Clarkson B, Strife A. Discordant maturation in chronic myelogenous leukemia. In: Deisseroth AB, Arlinghaus RB, eds. Chronic myelogenous leukemia—molecular approaches to research and therapy. New York: Marcel Dekker, 1990; 3–90.

Advances in Treatment for Hodgkin's Disease

PETER H. WIERNIK, M.D.

HODGKIN'S DISEASE (HD) is a good example of what other contributors to this symposium have discussed: that it is possible to make observations in the clinic that are important enough to lead to new scientific understandings about clinical problems and allow for new treatments to be developed.

Radiotherapy. The story of HD therapy began in the mid 1930s with Gilbert demonstrating that the disease was in fact amenable to radiotherapy, using equipment available at the time (1). It was in 1963 that Easson from Manchester published a paper about cancer with the word "cure" in the title—"The cure of Hodgkin's disease" (2)—the first such use I am aware of. Now, with refinement of radiotherapy technique and the development of megavoltage equipment, one can actually cure the majority of patients who have HD confined to lymph nodes.

Chemotherapy. At about the time I was graduating from medical school, it also became apparent that chemotherapy was effective against the disease. Not only were agents such as nitrogen mustard producing remissions, but Mortimer Lacher and others like him were producing what appeared to be complete remissions with combinations of single agents (3). I suppose it was the work of Lacher and others which led to more extensive development of combination chemotherapy for HD. It took long hours of work and many individuals involved along the way. The work culminated in the MOPP program, which was developed in Bethesda by DeVita and colleagues (4). As a first-year fellow, I had the privilege of entering patients on that study in Baltimore, so I remember with astonishment patients with advanced disease "cured" with combinations of active agents that were sensibly put together after clinical trial and error. The MOPP program was apparently "curing" patients, certainly producing disease-free survivals of substantial duration and possibly "cure." We know now that cure was in fact the rule rather than the exception for asymptomatic patients with extensive disease limited to lymph nodes.

Combined Modality Therapy. I became interested in patients who had Hodgkin's disease and as a third-year fellow began to study combinations of radiotherapy and chemotherapy as initial treatment for these patients. Neither my mentor, Arthur Serpick, nor I knew that similar work was going on in many places; all of these studies eventually produced similar results. In general, combined-modality therapy for HD confined to lymph nodes was superior to radiation therapy (5). In some studies the results of comparisons of treatments were not statistically significantly different, although in most studies, combined modality therapy appeared to have a benefit. Eventually, studies at Stanford, at the Baltimore Cancer Research Center, and at a number of sites in Europe made clear that disease-free survival was enhanced by combinations of radiotherapy and chemotherapy, even in patients with early-stage disease, although overall survival differences were not always significantly different. The explanation that some gave was that salvage therapy, which I believe is a misnomer, was so effective for

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. From the Division of Medical Oncology, Department of Medicine, Albert Einstein College of Medicine, Bronx. Address reprint requests to the author, Guttman Professor and Chairman, Department of Oncology, Montefiore Medical Center, 111 East 210th Street, Bronx, NY 10467.

early-stage patients that a relapse did not really matter. One could retreat and achieve the same overall survival.

Identifying Subsets of Patients. We began to look at prognostic factors in HD patients to determine whether or not we could decipher groups of patients who might benefit from combined-modality therapy and groups of patients who might not. We were the first to report that one group, patients with large mediastinal masses, benefited from combined modality therapy, and that they had better disease-free and overall survival than patients treated with radiotherapy alone (6, 7). The results were statistically significant and clearly in favor of the combined modality treatment. This observation was not readily accepted by some groups, but I think it is fair to say that basically everyone has accepted that large mediastinal masses confer poor prognosis for cure with radiotherapy alone, and that results are significantly better with combined modality therapy.

Another subset of patients found to benefit from combined modality therapy was patients with extensive intraabdominal disease, stage IIIA disease with involvement beyond the upper abdomen, spleen, splenic portal nodes, porta hepatis nodes, or high epigastric nodes (8–12). These findings were reported early by Desser in Chicago (10), a group at Vanderbilt, and our own group (11). In fact, in our experience, only 50% of patients with stage IIIA disease could be cured with radiotherapy when they had extensive intraabdominal disease. Those results were initially considered to be poorer than one might expect, but in fact the Joint Center at Harvard, the Stanford group, and others have published data that were almost identical to the Baltimore data. I think it is generally accepted that extensive intraabdominal disease (Stage $IIIA_2$) is a poor prognostic presentation for potential cure with radiotherapy alone.

Using Combination Chemotherapy Alone. The question then became, "Do you need the radiotherapy?" The MOPP regimen was curing patients with advanced disease—stages IIIB, IVA, IVB—and it would be strange indeed if curing less extensive disease with the same treatment were more difficult. So the reasonable expectation was that MOPP might be efficacious in early-stage disease, and that combination chemotherapy added to radiotherapy might not be superior to chemotherapy alone. We put that to the test in 1976 in a pilot study that posed difficult ethical and administrative questions. From our small pilot study we have been able to follow patients continuously to the present day (13). The results show no difference in outcome between MOPP combination chemotherapy or radiotherapy followed by MOPP combination chemotherapy for patients with early-stage disease as initial treatment, when these treatments were given as initial treatment for previously untreated patients.

That result suggested to us that combination chemotherapy alone might be of real value to patients with early-stage disease, and that the question deserved further study. Our rationale for pursuing this issue and the rationale, I think, of others who pursued it is that long-term results, especially long-term toxicity of treatment, might be significantly different for the two approaches.

One fact of concern is that most patients with HD who are cured with radiotherapy are young people, and we and others have noted an appreciable incidence of subclinical and clinical cardiac abnormalities years after completing mantle irradiation for HD. We noticed a significant incidence of hypothyroidism after radiotherapy which is, on the surface, a simple problem, but when one realizes that hypothyroidism accounts for about one third of the infertility that's been ascribed to HD and its treatment, it becomes a more significant problem. The occasional death of a young patient without any risk factors for coronary artery disease who at autopsy has intimal proliferation that ends precisely at the radiotherapy port edge begins to raise concern: 20 or 30 years from now, will this become more of a problem than it is now (14)?

So it seemed to me that although chemotherapy entails potential toxicities, it would be rational to determine whether chemotherapy could replace radiotherapy for early-stage HD. A study under the direction of Vincent DeVita, which I had a role in developing, was begun at the National Cancer Institute in 1978. The long-term follow-up data of that study are extremely interesting. In capsule summary, the difference in disease-free and overall survival of patients with early-stage HD treated with radiotherapy alone or with MOPP combination chemotherapy alone is minimal, and favors MOPP (15). Thus we now have two modalities that will cure the majority of patients with early-stage HD.

These advances in HD treatment have occurred because of the interactions of numerous investigators all around the world for less than a generation. Being an oncologist at this time is exciting. I learned to be excited from Jim Holland, and being excited has not yet let me down.

References

1. Gilbert R. Radiotherapy in Hodgkin's disease (malignant granulomatosis): anatomic and clinical foundations: governing principles: results. Am J Roentgenol 1939, 41:198–241.
2. Easson EC, Russell MH. The cure of Hodgkin's disease. Br Med J 1963; 1:1704.
3. Lacher MJ, Durant JR. Combined vinblastine and chlorambucil therapy of Hodgkin's disease. Ann Intern Med 1965, 62:468–476.
4. DeVita VT, Serpick AA, Carbone PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 1970; 73:891–895.
5. Mauch P, Goffman T, Rosenthal DS, et al. Stage III Hodgkin's disease: improved survival with combined modality therapy as compared with radiation therapy alone. J Clin Oncol 1985; 3:1166–1173.
6. Levi JA, Wiernik PH. Limited extranodal Hodgkin's disease: unfavorable prognosis and therapeutic implications. Am J Med 1977; 63:365–372.
7. Mauch P, Gorshein D, Cunningham A, et al. Influence of mediastinal adenopathy on site and frequency of relapse in patients with Hodgkin's disease. Cancer Treat Rep 1982; 66:809–817.
8. Stein RS, Golomb HM, Diggs CH, et al. Anatomic substages of stage IIIA Hodgkin's disease: a collaborative study. Ann Intern Med 1980; 92:149.
9. Stein RS, Golomb HM, Wiernik PH, et al. Anatomic substages of stage IIIA Hodgkin's disease: follow-up of a collaborative study. Cancer Treat Rep 1982; 66:733.
10. Desser RK, Golomb HM, Ultmann JE, et al. Prognostic classification of Hodgkin's disease in pathologic stage III, based on anatomic consideration. Blood 1977; 49: 883.
11. Levi JA, Wiernik PH, O'Connell MJ. Patterns of relapse in stages I, II and IIIA Hodgkin's disease: influence of initial therapy and implications for the future. Int J Rad Oncol Biol Biophys 1977; 2:853–862.
12. Hoppe RT, Cox RS, Rosenberg SA, Kaplan HS. Prognostic factors in pathologic stage III Hodgkin's disease. Cancer Treat Rep 1982; 66:743.
13. O'Dwyer PJ, Wiernik PH, Stewart MB, et al. Treatment of early stage Hodgkin's disease: a randomized trial of radiotherapy plus chemotherapy versus chemotherapy alone. In: Cavalli F, Bonadonna G, Rosenzweig N, eds. Malignant lymphomas in Hodgkin's disease: experimental and therapeutic advances. Boston: Martinus Nijhoff, 1985; 329–336.
14. Dollinger MR, Lavine DM, Foye LV. Myocardial infarction due to post irradiation fibrosis of the coronary arteries: case of successfully treated Hodgkin's disease with lower esophageal involvement. JAMA 1966; 195: 316.
15. Longo DL, Glatstein E, Duffey PL, et al. Radiation therapy versus combination chemotherapy in the treatment of early stage Hodgkin's disease: seven-year results of a prospective randomized trial. J Clin Oncol 1991; 9:906–917.

The Evolution of Curative Cancer Therapy

Emil Frei III, M.D.

I would like to describe an approach to curative cancer chemotherapy which derives from some well-known principles of dose, schedule, combination chemotherapy, and which I think can help make inroads against solid tumors. This approach is built around alkylating agents.

Non-Cross-Resistance of Alkylating Agents. Alkylating agents are heterogeneous drugs, and together with cisplatin, ingredients of almost all the current curative regimens. Ten to fifteen years ago, we were pretty certain that alkylating agents were all the same. There may be minor differences in dose and schedule, toxicity, and therapeutic spectrum. And they were believed to be cross-resistant. About ten years ago, the late Frank Schabel sent me some information on cross-resistance among alkylating agents in mouse L1210 leukemia cells (1). In his studies, he showed that a single dose of an alkylating agent produces a 5-to-7-log cell kill. By serial passage, Schabel produced a line resistant to cyclophosphamide. And, contrary to expectation, he found it not to be cross-resistant to other alkylating agents. He found the same situation with L-PAM, BCNU, cisplatin. So the dogma that there is almost always cross-resistance among alkylating agents was not correct, at least for mouse L1210 cells; it was antidogma, and an old chemotherapy warhorse like myself sat up and took notice, because if the agents are not cross-resistant with each other, they have all kinds of potential.

Because we had been fooled in the past, extrapolating from mouse to humans, we decided to produce resistance to alkylating agents in a number of human tumor cell lines (2). We exposed the cells to increasing concentrations, just short of killing the total population, of a chemotherapy agent over time. We then assayed the cells for drug resistance. Methotrexate or doxorubicin produced a hundred, a thousand, even ten-thousand-fold resistance. But the concentration of the alkylating agents could only be increased to a certain level beyond which all cells would be killed. At about the level of twenty-fold initial dose concentration, the cells were only 5-to-15-fold resistant to the alkylating agents. Obviously this "resistance" to development of resistance entails important qualities. We conducted experiments demonstrating that human tumor cell lines made resistant to one of the alkylating agents did not evidence cross-resistance to other alkylating agents (3). This confirmed Schabel's finding in a human tumor: cross-resistance was not common among the alkylating agents.

All aspects of alkylating agent metabolism and action relating to mechanisms of resistance, including transport, glutathione metabolism, DNA damage, and repair, have been described (4). In many instances, the diversity of resistance mechanisms explains the lack of cross-resistance. Some alkylating agents are actively transported within the cell, and resistance can occur by selecting cells that do not have this transport process. Within the cytoplasm, the alkylating agents face a sea of hostility. There are high concentrations of glutathione which can scavenge alkylating agents, particularly in the presence of glutathione-S-transferase. Once the alkylating agent has arrived intact in the nucleus, its DNA-damaging properties may be limited by hypoxia, which is common in solid tumors. This can be reversed by

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. From the Department of Medicine, Harvard University School of Medicine, Boston. Address reprint requests to the author, Director and Physician-in-Chief, Dana Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

fluosol, etanidazole, and perhaps pentoxyfylline. DNA damage by the alkylating agents can be repaired, thereby aborting cell kill. DNA repair can be inhibited by a variety of means. If these mechanisms of resistance can be modulated, the potential for therapeutic advantage may be increased.

Because of their non-cross-resistance to one another, alkylating agents could be used in combination. Schabel, following up on his observation, showed synergism among the alkylating agents in mouse leukemia.

Maximizing Dose. With increasing doses of alkylating agent against MCF-7 breast carcinoma cells, a straight-line cell-kill log-linear curve is produced. This is similar to radiation, which also produces a straight line. For instance, a fourfold increase in dose produces a four-log increase in cell kill (one log cell kill translates to good clinical partial remission, but a cure requires multilog cell kill). Vincristine, Ara-C, and many of the antimetabolites have a curvilinear plot of dose against cell kill, with only a 2- or 3-log cell kill no matter how high the dose. The curvilinear plot is largely due to resistant cell populations. Alkylating agents are therefore better drugs if multilog cell kill is desired.

Combination Chemotherapy. The most compelling evidence for dose is the transplantation experience, where the dose of alkylating agents can be given up to twentyfold with autologous bone marrow transplantation before dose-limiting nonmyelosuppressive toxicity occurs. Since the nonmyelosuppressive toxicities are often qualitatively different for each alkylating agent, this permits their use in combination. In the Johns Hopkins experience, cyclophosphamide and busulfan are individually relatively ineffective in the treatment of acute myelocytic leukemia. But when given in combination in the transplant setting to patients in first complete remission, the combination achieved a 50% cure rate, demonstrating a steep dose-response curve.

About six or seven years ago, William Peters and I put together a program of cyclophosphamide, cisplatin, and BCNU, and escalated the doses with autologous bone marrow transplantation. In this phase I study in patients with advanced refractory solid tumors, we found some gratifying responses (5). For example, patients with previously treated metastatic breast cancer had high response rates and a respectable complete response rate. And so it was with other tumors, with an overall 80% to 90% response rate. We concluded that the combination of cyclophosphamide, BCNU, and cisplatin was highly active in solid tumors. The bad news was that remissions were usually brief, and toxicity was substantial; mortality was 15%.

BCNU is a troublesome drug, produces visceral toxicity, and has variable pharmacokinetics, which may explain major toxicity in some patients and no effect in others. Cisplatin is not an ideal drug for bone marrow transplantation since the dose cannot be escalated because of renal toxicity and neurotoxicities. On the other hand, carboplatin is therapeutically as active as cisplatin, and, since its dose-limiting toxicity is myelosuppression, could be escalated from 400 mg to 2 g with bone marrow transplantation, a fivefold increase. Thiotepa is as active as a single agent in breast cancer as BCNU, and was less toxic.

After phase I studies of carboplatin and thiotepa individually in the transplant arena, we looked a cyclophosphamide plus thiotepa and finally cyclophosphamide plus thiotepa plus carboplatin. Using three drugs seemed logical because there is no cross resistance. Tumors which are cured with chemotherapy almost always require a combination of at least three drugs. We are now treating patients initially with newly diagnosed metastatic breast cancer. We used induction therapy with Adriamycin-methotrexate-fluorouracil (AMF) to minimize the tumor burden, then delivered the CTCb (cyclophosphamide-thiotepa-carboplatin) combination in the transplant arena. The patients were then restaged. If they were in partial or complete remission, they went on to the intensification transplant therapy. If they had no response, they went off study. In the 29 patients in our initial Phase II study, there was one death (3%) (6). The best-responding patients should be those in complete remission with induction and complete remission after transplantation. There were 10 such patients, and 5 continued free of disease at follow-up of 1 to 3 years. For those who achieved only partial response with induction, 3 went on to complete response with transplantation. Another group of 4 patients had complete response by all criteria save bone, and the bone lesions were thought to be in the process of healing. For the patients who achieved only partial response, one would expect relapse, and that did occur. Of 12 patients, all but 2 have relapsed. The 16 patients who were in complete or near-complete remission have median disease-free survival of 19 months (6). The survival cure for the patients who achieved only partial remission is relatively poor. We are continuing to add new patients to the study, and follow them for longer periods of time to see if the plateau in the survival curve of complete responders can be maintained.

Modulation of Alkylating Agents. Since a great deal is known about the biochemistry of sensitivity and resistance to alkylating agents, is modulation possible? Modulation approaches to cancer chemotherapy are not new, but what is new is that modulation can be successful (4). The modulation of fluorouracil by leucovorin has clearly been successful in patients with colorectal carcinoma and probably successful in patients who have head, neck, or breast cancer.

One of the areas I discussed above was the cytoplasmic concentration of glutathione (GSH) and glutathione-S-transferase (GST). Glutathione inactivates alkylating agents by conjugation, mediated by the enzyme glutathione-S-transferase. A glutathione synthesizing enzyme can be inhibited by an antimetabolite buthionine sulfoximine (BSO). In experimental tumors BSO can decrease the concentration of glutathione by one log, that is by 90%, and can increase sensitivity or reverse resistance to alkylating agents (7). Glutathione-S-transferase can also be inhibited by ethacrynic acid (8).

Another area for modulation of alkylating agents is in tumor hypoxia. Tumor cells that are more than 150 microns from a blood vessel or capillary are beyond the diffusing capacity of oxygen. Therefore there is a gradient of hypoxia where cells are not dead but resting and not subject to many of the agents, including x-ray, which require oxygen for mediation of cytotoxicity.

One approach to correcting that hypoxia is the use of fluosol. Fluosol is a perfluorocarbon. Tumors are hypovascular and hypoxic. Fluosol can onload and offload oxygen and carbon dioxide and is therefore a red blood cell substitute. The important thing about fluosol, however, is that its particle size is less than 1% of that of the red cell. Because of size, it has been clearly demonstrated that it can get into relatively hypoxic, that is hypovascular, tumors. Under these circumstances it enters hypovascular tumors and corrects hypoxia.

Fluosol can increase the therapeutic effect not only of x-ray, but also of a number of chemotherapeutic agents. Teicher looked at growth delay in Lewis lung tumor xenografts treated with radiation at increasing doses in combination with fluosol plus oxygen. There was a four- to five-fold increase in growth delay at 10 Gy and also at 20 Gy when fluosol and oxygen were added. At 30 Gy, a fairly marked increase occurred, presumably due to correcting the hypoxia in these experimental solid tumors (9). Teicher looked at a number of chemotherapeutic agents, including alkylating agents, and found a two- to five- or six-fold advantage if fluosol and oxygen were delivered (10). And it can be demonstrated that the partial pressure of oxygen is altered in tumors by direct studies. Tumors are generally 70%–80% hypoxic. With fluosol, the proportion of cells in tumors that are markedly hypoxic is much decreased (11). Other drugs such as etanidazole and pentoxyfylline can also partially correct hypoxia or act as oxygen mimics.

The activity of alkylating agents can also be modulated by inhibiting DNA repair of damage produced by the drugs. The function of the topoisomerases I and II is to change the topology of DNA, which is tightly bound during most of the cell cycle but has to loosen up during G2, the latter part of the S phase, and the mitotic phase to allow for DNA reading out, to repair DNA, contact with enzymes, and so on. In any event, topoisomerase II binds to a DNA strand at specific sequence sites, breaks the DNA strand, and allows for DNA passage, unwinding, and reading. Under normal circumstances, the DNA is resealed, and the cycles repeat. A number of agents can inhibit topoisomerase II. Indeed, low topoisomerase II levels have been found to be one of the mechanisms for multidrug resistance, the other two being the p-170 glycoprotein and glutathione-S-transferase. Novobiocin is an antibiotic which is not a cytotoxic agent but is an inhibitor of topoisomerase II. Novobiocin is inactive in growth delay studies, while cisplatin has some activity. Using the two together at full doses markedly increases growth delay (12). BCNU alone has a small effect; BCNU in combination with novobiocin has about a twelvefold effect over the control. This demonstrates that inhibition of topoisomerase II can increase the activity of alkylating agents, probably by decreasing the capacity for DNA repair.

Resistance to alkylating agents is 5-to-15-fold. Drug resistance, we found, is multifactorial more often than monofactorial. For example, a head and neck squamous cell carcinoma line that is fifteenfold resistant to cisplatin has a threefold decrease in transport, a two-and-a-half-fold increase in glutathione-S-transferase, and about a threefold increase in metallothionine. Thus three mechanisms can, in combination at relatively low levels, cause a fifteenfold resistance. Modulation of only one mechanism may therefore not result in much of an effect. Several mechanisms may need to be modulated to produce enhancement in clinical activity. Indeed, combinations of modulators on which we are conducting major experiments have shown efficacy. Cyclophosphamide plus fluosol-oxygen or cyclophosphamide plus etanidazole gives a partial improvement over cyclophosphamide alone in studies with fibrosarcoma in mice. With etanidazole and fluosol-oxygen together added to cyclophosphamide, a further log-cell kill can be obtained without increasing toxicity (13).

Our current combination for patients with stage II breast cancer is CTCb. Toxicity is low and response rates gratifyingly high. Patients with partial remissions have early relapse. Some of those with complete remissions have sustained early relapse, but a substantial fraction have continued in complete remission for two to three years now. We are going to continue with the CTCb combination, attempt to improve it, and make it safer by the use of hematopoietins and peripheral blood stem cells.

Many of the programs I have discussed derive from the principles of chemotherapy discovered by Jim Holland, such as the use of agents which are not cross-resistant in combination, maximizing dose, and treating patients with minimal or early disease wherever possible. We hope that these programs will be as successful as some of those Jim has pioneered over the years and is currently pioneering.

References

1. Schabel FM Jr, Trader MW, Laster WR Jr, Wheeler GP, Witt MH. Patterns of resistance and therapeutic synergism among alkylating agents. Antibiot Chemother 1978; 23:200.
2. Teicher BA, Frei E, III. Development of alkylating agent-resistant human tumor cell lines. Cancer Chemother Pharmacol 1988; 21:292–298.
3. Frei E III, Teicher BA, Holden SA, Cathcart KN, Wang YY. Preclinical studies and clinical correlation of the effect of alkylating agents. Cancer Res 1988; 48:6417–6423.
4. Frei E III. The modulation of alkylating agents. Semin Hematol 1991; 28:22–24.
5. Peters WP, Eder JP, Henner WD, et al. High dose combination alkylating agents with autologous bone marrow support: a phase I trial. J Clin Oncol 1986; 4:646–654.
6. Antman K, Ayash L, Elias A, et al. A Phase II study of high-dose cyclophosphamate thiotepa and carboplatin with autologous marrow support in women with measurable advanced breast cancer responding to standard dose therapy. J Clin Oncol 1992; 10:102–110.
7. Ford JM, Hait WM, Matlin SA, Benz CC. Modulation of resistance to alkylating agents in cancer cell by gossypol enantiomers. Cancer Lett 1991; 56:85–94.
8. Tew KD, Bomber AM, Hoffman SJ. Ethacrynic acid and piripost as enhancers of cytotoxicity in drug resistant and sensitive cell lines. Cancer Res 1988; 48:3622–3655.
9. Teicher BA, Rose CM. Sensitization of solid mouse tumor to x-ray treatment by oxygen-carrying perfluorochemical emulsion. Cancer Res 1984; 44:4285–4288.
10. Teicher BA, Holden SA. A survey of the effect of adding Fluosol-DA 20%/O2 to treatment with various chemotherapeutic agents. Cancer Treat Rep 1987; 71:173–177.
11. Song CW. Increase in p02 and radiosensitivity of tumors to Fluosol-DA (20%) and carbogen. Cancer Res 1987; 47:442–446.
12. Eder JP, Teicher BA, Holden SA, Cathcart KN, Schnipper LE, Frei E, III. Effect of novobiocin on the antitumor activity and tumor cell and bone marrow survivals of three alkylating agents. Cancer Res 1989; 49:595.
13. Teicher BA, Herman TS, Tanaka J, et al. Modulation of alkylating agents by etanidazole and Fluosol-DA/carbogen in the FSaIIC fibrosarcoma and EMT6 mammary carcinoma. Cancer Res 1991; 51:1986–1991.

The Past—Prelude to the Future

Ezra M. Greenspan, M.D.

I first met young Jim Holland quietly looking and listening over my shoulder while on rounds in 1952—in the days when cancer was "precurable"—at Francis Delafield Hospital. Jim was a resident-fellow at the Columbia-Presbyterian Cancer Facility, and I had just returned from the National Cancer Institute to The Mount Sinai Hospital and was a visiting attending at Delafield. I was full of enthusiasm for combination chemotherapy with thiotepa and methotrexate, modulation of methotrexate with timed folic acid and leucovorin, and had even begun a triple therapeutic combination with the potent podophyllin derivative, alpha peltatin, now extinct.

The generous free-wheeling canonization of the National Cancer Institute under Bo Mider and Harry Eagle had permitted me to return to New York. I attempted to set up something of a cancer program at The Mount Sinai Hospital, which had an unsympathetic and skeptical staff and no medical school at that time. In the next few years, I focused on treating breast and ovarian cancer with increasingly aggressive polychemotherapy. In the early fifties, enthusiasm was high, and ideas were as always cheap and easy. But it has been in the execution of ideas, the affirmation and extension of therapeutic concepts, in which Jim Holland has made a major impact in oncology during the last three decades. Not only did he and his colleagues—Frei, Freireich, and many others—establish the curability of the leukemias and the lymphomas; this group also established the controlled clinical trial as a basis for large cooperative group trials, which remain the bulwark of cancer chemotherapy in the United States today. This was a pioneering effort, and Jim was the essential catalyst, gadfly, critic, driver, and master of logistics pursuing the goal with a relentless energy. With his student Richard Cooper and his young colleagues Douglas Tormey and Larry Norton, among others, he inspired the Cancer and Leukemia Group B to prove that the intensive polychemotherapy of breast cancer could open the window of cure for that all-too-common fatal disease.

But with the controlled clinical trials came the dominance of statisticians who, while reinforcing the cooperative groups and group studies, also befuddled us at times. Arguments developed between the splitters and the lumpers, between the advocates of subgroup stratification and the advocates of meta-analysis, and these arguments continue to plague us, particularly on meta-analysis. Despite the 85% response rate in breast cancer, many question whether breast cancer is curable at all by chemotherapy. Meta-analysis shows only a 4%–8% benefit. Yet we know that 28% of breast cancer patients could be added to the cured roster with optimal adjuvant chemohormonal therapy today. Meta-analysis summarizes all trials, good, bad, or indifferent, and by lumping them together like a goulash, derives an erroneous conception of clinical chemotherapy.

Despite the 85% regression rate in breast cancer, why do only a minority respond with cure? This, as I see it, is the major challenge for us in the next decade. Unfortunately, the development of new cytotoxic agents has slowed, but fortunately they are being supplemented by a return to biological, biochemical, and immune modula-

---

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" commemorating the 65th birthday of James F. Holland at the Mount Sinai Medical Center on May 29, 1990. From the Division of Medical Oncology, Department of Medicine, Mount Sinai School of Medicine, New York. Address reprint requests to the author, Clinical Professor of Medicine and Associate Chief, Division of Medical Oncology, Box 1178, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

tion. Genetic engineering has vastly expanded our potential armamentarium. Indeed, more modulators than cytotoxic agents are in trials now. In the new field of modulation, too much or too little of a good thing may be bad, and the optimal timing for treatment is critical and often evanescent. Newer statistical and clinical requirements are needed for the Food and Drug Administration to revise and liberalize its standards of efficacy so that 15% differences will be acceptable. Combinations of modulating agents will probably be necessary to evoke responses of greater magnitude.

We have to look to the host–patient relationship and the patient's individual genetic and immunological makeup as well as to tumor characteristics to predict and analyze who will respond and why some will be cured. As a clinician, I find it curious that some patients seem to be programmed to respond favorably to curable cytotoxic chemotherapy. In my clinical experience, a good programmer for the individual patient is successful recovery from previous granulomatous infection, especially tuberculosis. Perhaps we should paraphrase George Bernard Shaw after all: "The clues to success (cure) or failure may not be in our stars, or in our scans, or in our pills, but in our lymphocytes. Or was it the macrophages? Yes! It was the macrophages!"

Successful Successive Studies in Leukemia and Breast Cancer

JAMES F. HOLLAND, M.D.

I WAS ENORMOUSLY GRATIFIED by the company of eleven who addressed the symposium. To stand among them was an honor. I do want to thank Takao Ohnuma for remembering my birthday. He knew my anniversary was coming because his birthday is the same day as mine; he's not 65 or the party would have been for him. I look at all of those published in this issue with appreciation. I count myself a brother-in-arms with them because we have battled these things together. I've learned from my seniors and from the trailblazers and pathfinders at this symposium. And I. B. Weinstein is right. Both of us were effectively imprinted by Alfred Gellhorn. Imprinting is a two-way street: those who've learned teach, and those who are the taught teach us in return. And I was privileged to have a substantial number of my intellectual progeny, as well as my biological progeny and my coproducer, my wife, present at this symposium.

I am grateful to those who have supported our research over the years: Mr. and Mrs. Derald Ruttenberg, who have funded the Derald H. Ruttenberg Cancer Center, Mr. Tony Martell and Mr. Floyd Glinert, the President and Chairman of the Board of the T. J. Martell Foundation, who have supported our research, and many others who are colleagues and coparticipants in our research. The two figures are easily recognizable to them.

The top line of Fig. 1, the effect of treatment on childhood acute lymphoblastic leukemia, is not finished. The top line represents an early analysis of the last group of children on a study program that began in 1976; because of a concatenation of impediments, the last time those data were analyzed was 1980. A delayed analysis is now under way.

Dr. Frei's brilliant presentation focused on advanced breast cancer. My colleagues and I have directed our efforts to preventing relapse in breast cancer patients after mastectomy. Fig. 2 shows the results of adjuvant chemotherapy in women with breast cancer who have metastases to their axillary lymph nodes at the time of surgery. The latest program of treatment is named here in the vernacular "Cooper-Adria" because Richard Cooper, in a brilliant stroke as a practitioner, put together the five drugs that made a major impact on breast cancer. Jones, Norton, Bhardwaj, and I found the efficacy of intensive

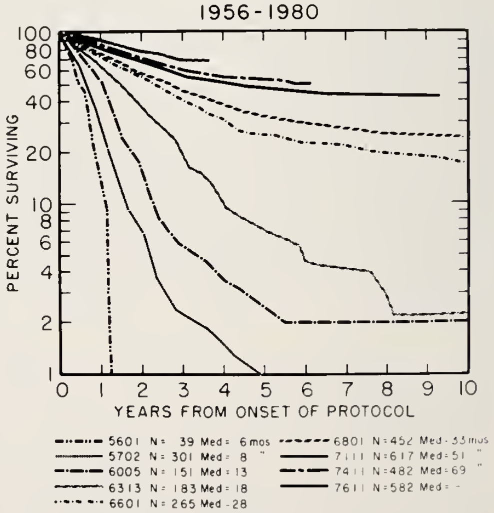


Fig. 1. Cancer and Leukemia Group B: acute lymphocytic leukemia survival in children under 20.

Adapted from the author's presentation at the symposium "Human Cancer: From 'Precurable' to Curable" at the Mount Sinai Medical Center on May 29, 1990. From the Derald H. Ruttenberg Cancer Center at the Mount Sinai Medical Center, New York. Address reprint requests to the author, who is Director of the Derald H. Ruttenberg Cancer Center at Mount Sinai and Jane B. and Jack R. Aron Professor and Chairman, Department of Neoplastic Diseases, at Box 1129, Gustave L. Levy Place, New York, NY 10029.

Adriamycin dosing in metastatic breast cancer. Bhardwaj, Norton and I put together an adjuvant therapy program using the Cooper regimen followed by intensive Adriamycin. Twenty-two patients with axillary-node-positive breast cancer were treated with this regimen. Only one woman has relapsed, and the observations extend for as long as six years. We think this is an important advance. It is the fifth regimen that we have studied. The other four led to progressively longer disease-free survival and begin to resemble the acute leukemia spectrum of gradually increasing activity. We have confirmed the data in another study of 47 women conducted in the Cancer and Leukemia Group B, where the results are nearly identical although for a shorter period of time. If these data are sustained, we may be able to keep Dr. Frei from having to look at therapies for women with advanced breast cancer.

That will require that I stay at work, which I fully intend to do. But I am a prudent man. I have squirreled away a little money for alternative support. I have found a little spot outside Dublin where a wee nip of convivial beverage can be had for therapeutic purposes. Right next door is a barber shop. In case chemotherapy goes out of style, I could revert to being a surgeon like the original barbers (Fig. 3).

JIM HOLLAND
Velvet Bar

Fig. 3. The "little spot outside Dublin," whose proprietor also happily bears the name Jim Holland.

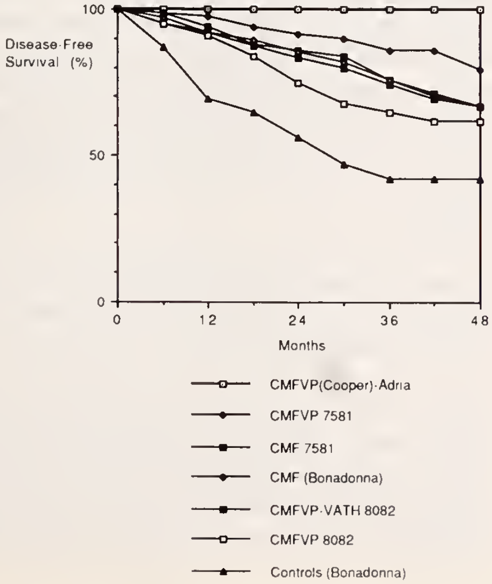


Fig. 2. Adjuvant chemotherapy of premenopausal patients with breast cancer, four or more axillary nodes.

Tamoxifen and Endometrial Cancer

RUDY A. SEGNA, M.D., PETER R. DOTTINO, M.D., LIANE DELIGDISCH, M.D., AND CARMEL J. COHEN, M.D.

Abstract

Tamoxifen is a nonsteroidal antiestrogen employed frequently in the treatment of breast cancer. An association between this drug and endometrial neoplasia has been reported. We report on 11 postmenopausal women with breast cancer who developed endometrial cancer while undergoing tamoxifen therapy and recommend aggressive investigation of vaginal bleeding in all women being treated with this agent.

INCREASINGLY, endometrial cancer is being reported in women receiving tamoxifen as part of their treatment for breast cancer (1–4). Breast cancer is the most common malignancy in women, and endometrial cancer is the most common gynecologic malignancy. Fornander et al. found a 6.5-fold higher occurrence of endometrial cancer in women with breast cancer receiving tamoxifen than in those with breast cancer not receiving this agent (1). Use of tamoxifen has become so common that any carcinogenic potential requires evaluation. Whether this drug itself is carcinogenic or acts by affecting an endometrium inherently programmed for neoplastic transformation is unknown. We describe 11 patients who developed endometrial cancer while receiving tamoxifen.

Case Reports. The 11 women in this series were all postmenopausal and taking tamoxifen for breast cancer (Table). All had postmenopausal uterine bleeding and, except for one patient taking megestrol acetate, none was receiving exogenous hormonal therapy. All patients had the diagnosis of endometrial cancer made by office biopsy or by dilatation and curettage. They were subsequently treated by total abdominal hysterectomy, bilateral salpingoophorectomy, and pelvic lymph node sampling between 1990 and 1992 by different clinicians. All pathologic specimens were reviewed by one of the authors (LD). Pathologic findings are summarized in the Table.

Discussion. Tamoxifen is a nonsteroidal antiestrogen which can have estrogenic properties. Its mechanism of action on the endometrium is not well understood. The role of unopposed estrogen intake in the development of endometrial cancer and its precursors is well established (5, 6). Like estradiol, tamoxifen binds to cytoplasmic estrogen receptors. This complex is then transferred to the nucleus and diminishes the number of unbound estrogen receptors, preventing binding by stronger estrogens and resulting in an antiestrogen effect. However, the effects of tamoxifen are much more complex than simple competitive inhibition.

The agonist and antagonist activity of tamoxifen can be seen in the nude mouse model. The agent has been shown to stimulate the growth of human endometrial cancer implanted in nude mice, but not as much as estradiol, while inhibiting the growth of estrogen-receptor-positive breast cancer inplanted in the same mouse (7). Other estrogenic effects of tamoxifen displayed in the nude mouse model include an increase in the progesterone receptor level (8) and the develop-

---

From the Department of Obstetrics, Gynecology and Reproductive Science, Division of Gynecologic Oncology, and the Department of Pathology (LD), Mount Sinai School of Medicine.

Address all correspondence to: Rudy A. Segna, M.D., Naval Hospital, Department of OB/GYN, Portsmouth, VA 23708. The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government.

Address reprint requests to Carmel J. Cohen, M.D., Department of OB/GYN, Box 1170, The Mount Sinai Hospital, One Gustave L. Levy Place, New York, NY 10029.

TABLE

Clinical Characteristics and Pathological Findings, 11 Postmenopausal Women with Breast Cancer Taking Tamoxifen (TMX)

Pt	Age	Symptom	TMX Rx	Uterine Pathology	Associated Changes
1	52	P.M.B.	20 mg/day for 5 mos	Poorly differentiated adenocarcinoma with invasion to middle third of myometrium	Adenomatous hyperplasia and adenomyosis
2	79	P.M.B.	20 mg/day for 18 mos	Moderately differentiated adenocarcinoma with no myometrial invasion	Adenomyosis; cystic and adenomatous hyperplasia endometrial polyps; leiomyoma
3	69	P.M.B.	20 mg/day for 2 yrs	Moderately differentiated adenocarcinoma with invasion to outer third of myometrium and vascular space involvement	Decidualization
4	65	P.M.B.	20 mg/day for 2 yrs	Moderately differentiated adenocarcinoma with no myometrial invasion	Cervical involvement
5	61	P.M.B.	10 mg/day for 3 yrs	Well differentiated adenocarcinoma with no myometrial invasion	Leiomyoma; adenomyosis
6	56	P.M.B.	20 mg/day for 3 yrs	Well differentiated adenocarcinoma with no myometrial invasion	Cystic glandular hyperplasia; adenomyosis; endometriosis
7	73	P.M.B.	10 mg/day for 4 yrs	Poorly differentiated adenocarcinoma with no myometrial invasion	Metastatic breast cancer to myometrium, parametrium, and paraaortic nodes
8	78	P.M.B.	20 mg/day for 4 yrs	Well differentiated adenocarcinoma with no myometrial invasion	Adenomyosis; cystic and adenomatous hyperplasia; sigmoid adenocarcinoma
9	52	P.M.B.	20 mg/day for 4 yrs	Well differentiated adenocarcinoma with no myometrial invasion	Cystic glandular hyperplasia; endometrial polyps; leiomyoma
10	52	P.M.B.	20 mg/day for 4 yrs	Well differentiated adenocarcinoma with no myometrial invasion	Cystic glandular hyperplasia; endometrial polyps; leiomyoma
11	69	P.M.B.	20 mg/day for 7 yrs	Moderately differentiated adenocarcinoma with invasion to inner third myometrium	Cystic and adenomatous hyperplasia; cervical polyp with hyperplastic glands; leiomyoma

PMB, postmenopausal bleeding.

ment of cystic glandular hyperplasia in the uterus (9).

Tamoxifen has been shown to be an effective adjuvant agent in the treatment of postmenopausal women with node-positive, estrogen-receptor-positive breast cancer. Its use in those with negative nodes remains controversial (10); some studies, however, do suggest a benefit in treating these patients (11). The optimal duration of therapy is also unspecified. Overall, however, the use of tamoxifen for prolonged periods of time in the treatment of breast cancer is becoming more widespread, and is likely to increase further because its use in healthy women with benign breast disease and those with risk factors for breast cancer is currently being evaluated (12, 13).

In 1985, Killackey et al. described three patients with endometrial cancer who were taking tamoxifen (2). The Swedish adjuvant tamoxifen study found an increased risk of endometrial cancer in patients receiving 40 mg/day (1). The Danish study revealed an increased risk in patients taking 30 mg/day (4). Our series describes the development of endometrial neoplasia in women taking 20 mg/day of tamoxifen; the results include a propensity for these lesions to occur in those receiving such therapy for more than 18 months.

Although the Swedish and Danish studies demonstrated the occurrence of "nonfatal" endometrial cancers in tamoxifen-treated patients, six patients in this series had moderately to poorly differentiated adenocarcinomas, a larger proportion of undifferentiated lesions than one would expect from cancer that results from unopposed estrogen stimulation. This finding corroborates that of Malfetano, who suggested that the endometrial cancers developing in women taking tamoxifen may be the more virulent (3). This may simply reflect the small number of patients in these series because physiologically, one cannot explain such a difference. The frequency of hyperplastic endometrium—7 out of 11 postmenopausal women—in our series supports the theory that tamoxifen's estrogen-like activity may lead to endometrial cancer and its precursors (14–16). Four of our patients had endometrial polyps on evaluation of their uterus, a finding that has previously been reported (17–18). An association between adenomyosis and tamoxifen therapy appears evident, as five of our patients had this finding.

Most authorities believe that the benefits of tamoxifen in the treatment of breast cancer outweigh the risks of developing endometrial cancer. Prospective studies involving the screening of patients taking this drug are required to further delineate its relationship to endometrial neoplasia. Vaginal bleeding in these women should not be ascribed to harmless hormonal stimulation, but should be investigated aggressively, beginning with an endometrial biopsy. Moreover, routine endometrial sampling in asymptomatic women taking tamoxifen may lead to early detection of endometrial cancer and its precursors, allowing for prompt therapeutic intervention. This routine sampling is especially important as clinical trials are in progress to evaluate the role of tamoxifen as prophylactic therapy in healthy women.

References

1. Fornander T, Rutqvist LE, Cedermark B, et al. Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. Lancet 1989; 1:117–120.
2. Killackey MA, Hakes TB, Pierce VR. Endometrial adenocarcinoma in breast cancer patients receiving anti-estrogens. Cancer Treat Rep 1985; 69:237–238.
3. Malfetano JH. Tamoxifen-associated endometrial carcinoma in postmenopausal breast cancer patients. Gynecol Oncol 1990; 39:82–84.
4. Andersson M, Storm HH, Mouridsen HT. Incidence of new primary cancers after adjuvant tamoxifen therapy and radiotherapy for early breast cancer. J Natl Cancer Inst 1991; 83:1013–1017.
5. Gusberg SB. Precursors of corpus carcinoma: estrogens and adenomatous hyperplasia. Am J Obstet Gynecol 1947; 54:905–907.
6. Ziehl H, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. N Engl J Med 1975; 293:1167–1170.
7. Gottardis MM, Robinson SP, Satyaswaroop PG, et al. Contrasting actions of tamoxifn on endometrial and breast tumor growth in the athymic mouse. Cancer Res 1988; 48:812–815.
8. Satyaswaroop PG, Zaino RJ, Mortel R. Estrogen-like effects of tamoxifen on human endometrial carcinoma transplanted into nude mice. Cancer Res 1984; 44: 4006–4010.
9. Martin L, Middleton E. Prolonged oestrogenic and mitogenic activity of tamoxifen in the ovariectomized mouse. J Endocr 1978; 78:125–129.
10. Buzdar AU. Current status of endocrine treatment of carcinoma of the breast. Semin Surg Oncol 1990; 6:77–82.
11. Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. Lancet 1992; 339:1–15, 71–85.
12. Davidson NE. Tamoxifen—panacea or Pandora's box? N Engl J Med 1992; 326:885–886.
13. Nayfield SG, Karp JE, Ford LG, et al. Potential role of tamoxifen in prevention of breast cancer. J Natl Cancer Inst 1991; 83:1450–1459.
14. Cross SS, Ismail SM. Endometrial hyperplasia in an oophorectomized woman receiving tamoxifen therapy. Case report. Br J Obstet Gynaecol 1990; 97:190–192.
15. Gal D, Kopel S, Bashevkin M, et al. Oncogenic potential of tamoxifen on endometria of postmenopausal women with breast cancer—preliminary report. Gynecol Oncol 1991; 42:120–123.
16. Nevan P, DeMuylder X, Van Belle Y, et al. Hysteroscopic follow-up during tamoxifen treatment. Eur J Obstet, Gynecol & Repro Biol 1990; 35:235–238.
17. Nuovo MA, Nuovo GJ, McCaffrey RM, et al. Endometrial polyps in postmenopausal patients receiving tamoxifen. Int J Gynecol Pathol 1989; 8:125–131.
18. Corley D, Rowe J, Curtis MT, et al. Postmenopausal bleeding from unusual endometrial polyps in women on chronic tamoxifen therapy. Obstet Gynecol 1992; 79: 111–116.

Submitted for publication May 1992.

N^{10}-propargyl-5,8-dideazafolic acid (CB3717):

Inhibitory Effects on Human Leukemia Cell Lines Resistant to Methotrexate or Trimetrexate

Yuruzu Takemura, M.D., and Takao Ohnuma, M.D., Ph.D.

Abstract

The inhibitory effects of N^{10}-propargyl-5,8-dideazafolic acid (CB3717), a quinazoline antifolate and a potent thymidylate synthase inhibitor, were evaluated in human leukemia cell lines resistant to methotrexate (MTX) and trimetrexate (TMQ). MTX-resistant MOLT-3 cell lines, MOLT-3/MTX$_{200}$ and MOLT-3/MTX$_{10,000}$, were cross-resistant to CB3717; however, the degree of resistance was only tenfold for both cell lines, and increased dihydrofolate reductase activity in MOLT-3/MTX$_{10,000}$ had little influence on the degree of CB3717 resistance. The MOLT-3 cell line made resistant to TMQ, MOLT-3/TMQ$_{200}$, was as sensitive to CB3717 as the parent line. The cell growth inhibitory effect of CB3717 on MOLT-3 was reversed by the addition of thymidine. Leucovorin also partially reversed CB3717-induced growth inhibition. Cellular uptake of MTX and 5-methyltetrahydrofolate was hindered by the presence of a high concentration of CB3717, whereas TMQ uptake was not influenced by CB3717. CB3717 appears to enter the cells not only through reduced folate transport system, but by other route(s). CB3717 does not share the transport pathway with TMQ. Our observations that MTX-resistant cells with increased dihydrofolate reductase are not more resistant than cells without increased enzyme activity, and that TMQ-resistant cells are not cross-resistant to CB3717, may have clinical relevance.

N^{10}-propargyl-5,8-dideazafolic acid (CB3717) is a unique quinazoline antifolate. Structurally it is of the propargyl group at N^{10} position of 2-amino-4-hydroxy-pteroyl-glutamate (1), which has been demonstrated to be an important site for thymidylate synthase (TS) inhibition (2–5). Although CB3717 has a weak inhibitory action on dihydrofolate reductase (DHFR), the major mechanism of its antitumor effect has been shown to depend on the inhibition of TS (6).

Clinical investigations and pharmacokinetic studies of CB3717 are in progress in Europe (7–12).

Recently, methotrexate (MTX)- and trimetrexate (TMQ)-resistant cell lines, designated MOLT-3/MTX$_{200}$, MOLT-3/MTX$_{10,000}$ and MOLT-3/TMQ$_{200}$, were developed in our laboratory from a human acute lymphoblastic leukemia (ALL) cell line, MOLT-3 (13–15). Resistance of MOLT-3/MTX$_{200}$ to MTX is related to its impaired membrane transport of the drug, whereas MOLT-3/MTX$_{10,000}$ has both impaired MTX transport and elevated DHFR activity (13, 14). MOLT-3/TMQ$_{200}$ has impaired membrane transport for TMQ and increased DHFR (15), though MTX transport is not impaired in this cell line. The TMQ-resistant cells also display characteristics of classic multidrug resistance (MDR) phenotype (15).

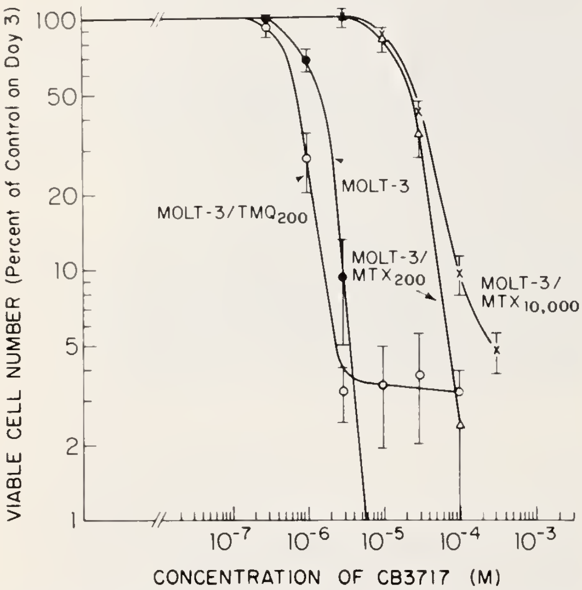
Supported in part by the Chemotherapy Foundation, Inc., New York, NY; by the United Leukemia Fund, New York, NY; and by the T. J. Martell Foundation for Leukemia and Cancer Research, New York, NY. Y.T. was an Iwama Memorial Sony International Fellow. From the Department of Neoplastic Diseases, Mount Sinai School of Medicine (CUNY), New York, NY. Address correspondence and reprint requests to Takao Ohnuma, M.D., Department of Neoplastic Diseases, Box 1128, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

To assess the role of this new antifolate CB3717 on folate resistance, we evaluated the inhibitory effects of this compound on these resistant sublines.

Materials and Methods

Cell Lines. The parent cell line, MOLT-3, was established from a patient with T-cell ALL (16) and has been maintained in our laboratory with RPMI-1640 medium (GIBCO, Grand Island, NY) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (GIBCO). Details of the development and properties of MTX- and TMQ-resistant sublines, MOLT-3/MTX$_{200}$, MOLT-3/MTX$_{10,000}$, and MOLT-3/TMQ$_{200}$, were described elsewhere (13–15). The experiments were performed after cells were fed with drug-free medium for at least two weeks. The cells in the exponential growth phase with >90% viability were used for all experiments.

Drugs and Radiomaterials. CB3717 is a white powder (ICI batch number ADM 59012/84, manufactured by ICI Pharmaceuticals, London), which was supplied courtesy of Professor K. Harrap, of the Institute of Cancer Research, Sutton, Surrey, UK. Since CB3717 was best dissolved at pH 9, stock solutions were prepared by dissolving the compound in 0.1 *N* NaOH. The solution of CB3717 in 0.1 *N* NaOH, protected from light and stored at −20°C, was stable for at least four weeks. The stock solution was sterilized by passing it through a 0.22-μm filter (Millipore Corp., Bedford, MA), neutralized with sterile 0.1 *N* HCl, and further diluted with Dulbecco's phosphate buffered saline (PBS, GIBCO) to the desired concentrations. MTX and leucovorin calcium (LV) were purchased from Lederle Laboratories



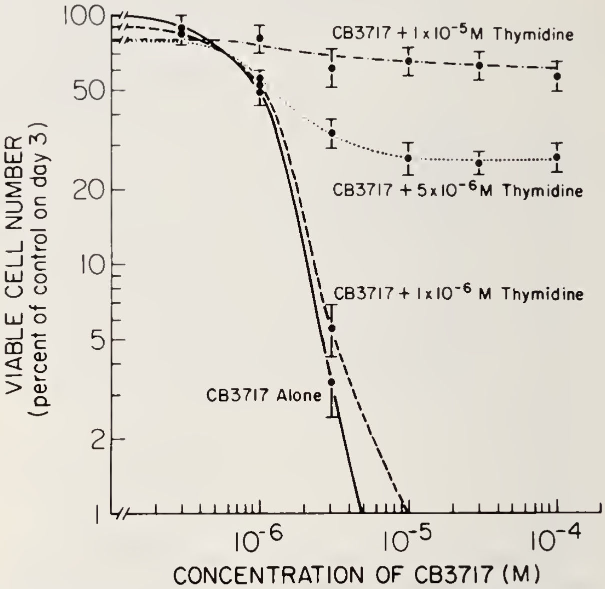


Fig. 1. **Left** Cell growth inhibitory effect of CB3717 on the parent and the resistant cell lines. Mean ± SD of five to seven triplicate experiments. **Fig. 2. Right** Reversal effect of thymidine on the growth inhibition of MOLT-3 cells by CB3717. Cells were continuously exposed to CB3717 and each concentration of thymidine for three days. Mean ± SD of two or more triplicate experiments.

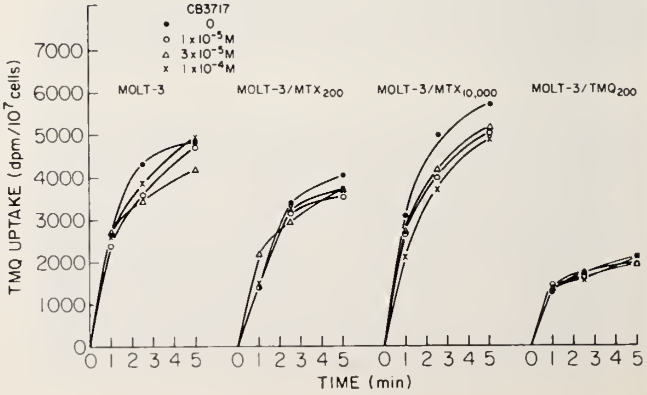


Fig. 3. Time course of [^{14}C]TMQ cellular uptake in the absence or presence of different concentrations of CB3717.

TABLE 1
Characteristics of Sublines of MOLT-3 Cells Resistant to MTX or TMQ

Cell lines	MTX or TMQ concentration (*M*) in culture medium	Cross-resistance/ collateral sensitivity		Doubling times (hr)	Drug transport		DHFR activity	Ref.
		MTX	TMQ		MTX	TMQ		
MOLT-3	0	×1	×1	18	—	—	18	14
MOLT-3/MTX$_{200}$	MTX, 5×10^{-6}	×200	×1/10	26	↓	NC	20	14
MOLT-3/MTX$_{10,000}$	MTX, 5×10^{-5}	×10,000	~×1/3	31	↓	NC	210	14
MOLT-3/TMQ$_{200}$	TMQ, 3×10^{-7}	×6	×200	22	NC	↓	76	15

NC, no change.
Relative resistance as calculated by taking ID$_{50}$ value of MOLT-3 as control. The downward arrows indicate the decreased membrane transport to the drugs, compared with the parent cells. DHFR activity was expressed as pmol of MTX bound per 10^8 cells (mean of assay done in triplicate, the range ±10% of the mean).

(Wayne, NJ). TMQ was obtained through the National Cancer Institute (Bethesda, MD). TMQ stock solution of 5×10^{-4} *M* was prepared by dissolving the compound in pure ethanol. It was stable for more than six months at 4°C (15). Thymidine was purchased from Sigma (St. Louis, MO). [^{3}H]MTX (10 Ci/mmol) and [^{14}C]methyl-tetrahydrofolate (THF) (58 mCi/mmol) were purchased from Amersham (Arlington Heights, IL). [^{14}C]TMQ (13.1 mCi/mmol) was supplied by Dr. R. Engle of the National Cancer Institute (Bethesda, MD).

Cell Growth Inhibition Assay. The degree of drug resistance was determined by a cell growth inhibition assay. Briefly, cell suspensions containing 1×10^5 viable cells/mL of RPMI-1640 medium supplemented with 10% FBS, graded concentrations of the drug with or without LV or thymidine were prepared in tissue culture tubes (Falcon 3033, Becton-Dickinson, Cockeysville, MD). After thorough mixing, 1-mL aliquots of cell suspensions were placed in a cell well (Corning 25820, Corning Glass Works, Corning, NY) and incubated at 37°C in humidified 5% CO_2–95% air atmosphere for three days in the continuous presence of the drug(s). At the end of the incubation period, viable cells were enumerated using the trypan blue dye exclusion method. The drug concentration–cell growth inhibition curves (dose-response curves) were drawn by plotting the number of viable cells as a percentage of the control against drug concentrations. The magnitude of resistance was expressed by the degree of displacement of the dose-response curve at ID$_{50}$ (50% inhibiting drug concentration for cell growth on day 3).

Cellular Uptake of [^{3}H]MTX, [^{14}C]Methyl-THF, and [^{14}C]TMQ in the Presence of CB3717. The influence of CB3717 on the initial uptake of radioactive MTX, methyl-THF, and TMQ was studied using the Vortex-Finnpipette procedure (17). Aliquots of 0.1 mL [^{3}H]MTX (10 μCi/mL, 1×10^{-6} *M*) or [^{14}C]methyl-THF (5 μCi/mL, 8.6×10^{-5} *M*), and 0.1 mL of graded concentrations of CB3717 or PBS were added to 1.8-mL volumes of cell suspensions (containing 2×10^7 cells) in Hank's balanced salt solution (HBSS, GIBCO) supplemented with 1 mg/mL bovine serum albumin (Sigma), and incubated at 25°C or 2°C. Pilot studies showed that cellular uptake of MTX and methyl-THF was linear for at least 5 min. For the measurement of [^{14}C]TMQ uptake, 2×10^7 cells in 1.8 mL of HBSS were incubated with 0.1 mL of [^{14}C]TMQ (1 μCi/mL, 7.6×10^{-5} *M*) and 0.1 mL of graded concentrations of CB3717 for 1, 2.5, or 5 min. At the end of the incubation period, 0.5-mL aliquots of reaction mixture were mounted on an oil layer (a mixture of mineral oil [Invenex Pharmaceuticals, Chagrin Falls, OH] and silicon oil [William F. Nye, New Bedford, MA] of which the final density was adjusted to 1.028 g/mL). The cells were separated from supernatant under the oil layer by centrifugation at 12,000 *g* for 30 sec using an Eppendorf microcentrifuge (Model 5412, Brinkmann Instruments, Westbury, NY).

After the supernatant and most of the oil layer were removed, 1 mL of deionized water was added to the cell pellet, thoroughly mixed, and transferred to a counting vial. Radioactivities in

TABLE 2
Reversal Effects of Equimolar Concentrations of Leucovorin (LV) on CB3717-Induced Growth Inhibition of Sensitive or Resistant MOLT-3 Cells

Cell lines	ID$_{50}$ (μM)		ID$_{80}$ (μM)	
	CB3717 alone	CB3717 + LV	CB3717 alone	CB3717 + LV
MOLT-3	1.9	4.7	2.6	34
MOLT-3/MTX$_{200}$	22	63	37	200
MOLT-3/MTX$_{10,000}$	24	90	56	270
MOLT-3/TMQ$_{200}$	0.72	5.9	1.1	60

TABLE 3

Cellular Uptake of [^{3}H]MTX and [^{14}C]Methyl-THF by the Parent and MTX or TMQ Resistant MOLT-3 Cells in the Presence of CB3717

	[^{3}H]MTX uptake at 2.5 min (dpm/10^7 cells) CB3717 concentration (μM)		[^{14}C]methyl-THF uptake at 2.5 min (dpm/10^7 cells) CB3717 concentration (μM)	
Cell lines	0	100	0	100
MOLT-3	14811 ± 1445	4382 ± 4163 (29.6)	1090 ± 201	795 ± 48 (72.9)
MOLT-3/MTX$_{200}$	5680 ± 1422	4443 ± 3924 (78.2)	723 ± 155	545 ± 168 (75.3)
MOLT-3/MTX$_{10,000}$	6658 ± 1749	5158 ± 3372 (77.5)	505 ± 82	475 ± 68 (93.7)
MOLT-3/TMQ$_{200}$	12626 ± 1920	4152 ± 1974 (32.9)	878 ± 13	623 ± 48 (70.9)

Values are expressed as a mean ± SD (uptake in the presence of 100 μM CB3717/uptake without CB3717 in percentage) from three separate experiments.

the cell pellet were determined by a liquid scintillation counter (Model LS-355, Beckman, Somerset, NJ). Counting efficiency was 70% for [^{14}C] and 40% for [^{3}H] after the addition of 10 mL of aqueous counting scintillant (ACS) (Amersham). The cellular uptake of each radioactive material at specified time intervals was calculated by subtracting the radioactivity at 2° from 25°C value.

Results

Resistance patterns of the MOLT-3 sublines to MTX and TMQ are summarized in Table 1. MOLT-3/TMQ$_{200}$ cells had as a whole approximately sixfold cross-resistance to MTX, reflecting the elevated DHFR levels (15). A small population of MOLT-3/TMQ$_{200}$ cells (less than 5%) was extremely resistant to MTX and could not be eliminated even in high concentrations of the drug. Both MTX-resistant sublines, MOLT-3/MTX$_{200}$ and MOLT-3/MTX$_{10,000}$, showed collateral sensitivities to TMQ, as previously reported (13, 14, 18). MOLT-3/MTX$_{10,000}$ also contained a small subpopulation of cells that were resistant to TMQ (14). Figure 1 shows that two MTX-resistant sublines are cross-resistant to CB3717; however, the degree of resistance was only tenfold in both sublines on the basis of ID$_{50}$ values. MOLT-3/TMQ$_{200}$ cells were sensitive to CB3717 in the similar degree to or slightly more than the parent MOLT-3 cells. A small population (3%) of the TMQ-resistant cells was again noted to be extremely resistant to CB3717.

Figure 2 shows the reversing effects of thymidine on CB3717-induced growth inhibition of MOLT-3 cells. In the simultaneous addition of 1×10^{-5} *M* of thymidine, a slightly toxic concentration for cell growth, the effect of CB3717 on MOLT-3 cells was completely reversed. As demonstrated in Table 2, LV also reversed the inhibitory effect of CB3717. Equimolar concentrations of LV were able to reverse CB3717-induced cell growth inhibition of the parent and TMQ-resistant cells more efficaciously than the MTX-resistant cells, which had impaired membrane transport for the drug (see ID$_{80}$ values).

The ability of CB3717 to influence cellular uptake of [^{3}H]MTX and [^{14}C]methyl-THF is shown in Table 3. As previously reported (14), cellular uptake of these two folates was decreased in the MTX-resistant sublines. In the presence of 1×10^{-4} *M* CB3717, there was a trend toward decreased uptake of MTX and methyl-THF in all cell lines tested. At lower concentrations of CB3717, no significant effects on MTX and methyl-THF uptake were observed. Furthermore, the uptake of [^{14}C]TMQ by the parent and MTX- or TMQ-resistant cells was not hindered by the presence of CB3717 at any concentrations tested (Fig. 3).

Discussion

In the present study, we demonstrated inhibitory effects of CB3717 on the sensitive parent MOLT-3 cells and those resistant to MTX or TMQ. This new drug was shown to be equally active against two types of MTX-resistant cells that had either normal or elevated levels of DHFR activities, indicating that elevated DHFR activity had little influence on the inhibitory effects of CB3717. This observation is consistent with reports that the major antitumor action of this drug is dependent on TS inhibition (1). In addition, both the 200- and 10,000-fold MTX-resistant cell lines were only tenfold cross-resistant to CB3717. This finding is also in accord with that of Diddens et al. (19), who studied the biologic effects of CB3717 on six sets of parent and MTX-resistant cell lines derived from various malignant tumor cells. Although there was cross-resistance to CB3717, the degree of resistance was markedly reduced in each set.

The TMQ-resistant cells have an increased DHFR activity and impaired membrane transport for TMQ but not for MTX (15). The majority of these cells were shown to be sensitive to CB3717 in the same degree as or in even slightly higher degree than the parent cells, while both MTX-resistant sublines demonstrated slight cross-resistances to CB3717. This observation should have important clinical implications. We observed about 3% of the cell population of MOLT-3/TMQ$_{200}$ cells to be extremely resistant to CB3717 (Fig. 1). We selected this subpopulation of the cells by pulsatile exposure to high concentrations of CB3717 (20); the new subline resistant to both TMQ and CB3717 was shown to have an increase in MDR1 mRNA with concomitant enhancement of multidrug resistance phenotype but neither increases in TS activity nor overexpressions of TS and DHFR mRNA, compared to those in MOLT-3/TMQ$_{200}$ cells. Since this unique subline was fifteenfold more cross-resistant to MTX than MOLT-3/TMQ$_{200}$, the possible mechanism of the common resistance to CB3717 and MTX seen in this subline seemed to be reduced polyglutamate formation rather than enzyme overproductions or transport defect (20). In fact, formation of polyglutamate metabolites can influence the cytotoxic actions of these antifolate drugs (21–24). The biochemical basis for the extreme resistance to MTX seen in the subpopulation of MOLT-3/TMQ$_{200}$ cells (15) can also be explained by the same interpretation.

We found that CB3717 was active against the TMQ-resistant cells that had impaired membrane TMQ transport and that radiolabeled TMQ entered the parent and resistant cells irrespective of the coexistence of CB3717. These observations indicate that CB3717 is differently transported into the cells from TMQ. The hypothesis that CB3717 shares a common transport system with MTX/reduced folates remains controversial (25, 26). The MTX-resistant L1210 cells that had the impaired membrane transport for MTX showed cross-resistance to CB3717; CB3717 also caused reductions of uptake of both MTX and folic acid in a dose-dependent manner (1). These findings suggest that CB3717 utilizes transport pathways for both MTX/reduced folates and folic acid. In contrast, [^{3}H]CB3717 uptake was reported not to be affected by the addition of a fourteenfold excess of either folic or folinic acid and seemed to occur by a nonsaturable process (25). Recently, it was demonstrated that CB3717 had high affinity for a membrane-associated folate-binding protein (FBP) (26). CB3717 appears to be transported by at least two routes: the classic reduced folate transport system, and a second route that may be associated with a high level of FBP. Our observation that a hundredfold greater concentration of CB3717 produced only a small inhibition of radioactive MTX uptake by the parent and resistant cells indicates little influence of the presence of CB3717 on the cellular uptake of MTX/reduced folate or a low affinity of CB3717 for MTX/reduced folate carrier protein. Although, owing to the lack of availability of the radiolabeled drug, we could not directly measure CB3717 uptake by our resistant cells, it is possible that CB3717 is transported into the cells predominantly by FBP and that the reduced folate transport systems play only a minor role in CB3717 transport. Our observation that the reversal effects of LV on CB3717-induced cell growth inhibition were less efficacious in the MTX-resistant cells than in the parent and TMQ-resistant cells also suggests that the resistant cells had impaired membrane transport for reduced folates but not for CB3717.

This observation that LV can reverse the growth inhibitory effects of CB3717 is difficult to explain, since DHFR is not a target for this compound. This may simply indicate that DHFR inhibition is not an insignificant mode of the activity of this compound. Alternatively, this partial reversal may be attributed to expansion of the intracellular methylenetetrahydrofolate pool and the competition of the substrate with the inhibitor (1).

In conclusion, CB3717 was shown to have no cross-resistance with TMQ-resistant cells. Virtually identical dose-response curves of the two MTX-resistant sublines with CB3717 indicate that CB3717 is active in MTX-resistant cells by virtue of elevated DHFR activity. Clinical MTX resistance is, in general, known to be associated with increases in DHFR rather than with decreased transport (18). CB3717 and analogs with similar mechanism of action might circumvent the resistance developed in patients treated with TMQ or MTX.

References

1. Jones TR, Calvert AH, Jackman AL, Brown SJ, Jones M, Harrap KR. A potent antitumour quinazoline inhibitor of thymidylate synthetase: synthesis, biological properties and therapeutic results in mice. Eur J Cancer 1981; 17:11–9.
2. Bird OD, Vaitkus JW, Clarke J. 2-Amino-4-hydroxyquinazolines as inhibitors of thymidylate synthetase. Mol Pharmacol 1970; 6:573–5.
3. Carlin SC, Rosenberg RN, VandeVenter L, Friedkin M. Quinazoline antifolates as inhibitors of growth, dihydrofolate reductase and thymidylate synthetase of mouse neuroblastoma cells in culture. Mol Pharmacol 1974; 10:194–203.

4. Calvert AH, Jones TR, Jackman AL, Harrap KR. 2-Amino-4-hydroxyquinazolines with dual metabolic foci in methotrexate resistant cells. Proc Am Assoc Cancer Res 1979; 20:24.
5. Calvert AH, Jones TR, Jackman AL, Brown SJ, Harrap KR. An approach to the design of antimetabolites active against cells resistant to conventional agents illustrated by quinazoline antifolates with N-10 substitution. In: Davis W, Harrap KR, Stathopoulos G, eds. Advances in tumour prevention, detection and characterization. Vol 5. Human cancer. Its characterization and treatment. Amsterdam: Excerpta Medica, 1980:272–83.
6. Jackson RC, Jackman AL, Calvert AH. Biochemical effects of a quinazoline inhibitor of thymidylate synthetase, *N*-(4-(*N*-((2-amino-4-hydroxy-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamic acid (CB3717), on human lymphoblastoid cells. Biochem Pharmacol 1983; 32:3783–90.
7. Calvert AH, Alison DL, Harland SJ, et al. Phase I studies with CB3717 (*N*-(4-(*N*-((2-amino-4-hydroxy-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamic acid. Br J Cancer 1983; 48:116–7.
8. Alison DL, Newell DR, Sessa C, et al. The clinical pharmacokinetics of the novel antifolate N^{10}-propargyl-5,8-dideazafolic acid (CB3717). Cancer Chemother Pharmacol 1985; 14:265–71.
9. Vest S, Bork E, Hansen HH. A phase I evaluation of N^{10}-propargyl-5,8-dideazafolic acid. Eur J Cancer Clin Oncol 1988; 24:201–4.
10. Cantwell BMJ, Macaulay V, Harris AL, et al. Phase II study of the antifolate N^{10}-propargyl-5,8-dideazafolic acid (CB3717) in advanced breast cancer. Eur J Cancer Clin Oncol 1988; 24:733–6.
11. Harding MJ, Cantwell BMJ, Milstead RAV, Harris AL, Kaye SB. Phase II study of the thymidylate synthetase inhibitor CB3717 (N^{10}-propargyl-5,8-dideazafolic acid) in colorectal cancer. Br J Cancer 1988; 57:628–9.
12. Calvert AH, Newell DR, Jackman AL, et al. Recent preclinical and clinical studies with the thymidylate synthase inhibitor N^{10}-propargyl-5,8-dideazafolic acid (CB3717). NCI Monogr 1987; 5:213–8.
13. Ohnoshi T, Ohnuma T, Takahashi I, Scanlon KJ, Kamen BA, Holland JF. Establishment of methotrexate-resistant human acute lymphoblastic leukemia cells in culture and effects of folate antagonists. Cancer Res 1982; 42:1655–60.
14. Ohnuma T, Lo RJ, Scanlon KJ, et al. Evolution of methotrexate resistance of human acute lymphoblastic leukemia cells in vitro. Cancer Res 1985; 45:1815–22.
15. Arkin H, Ohnuma T, Kamen BA, Holland JF, Vallabhajosula S. Multidrug resistance in a human leukemic cell line selected for resistance to trimetrexate. Cancer Res 1989; 49:6556–61.
16. Minowada J, Ohnuma T, Moore GE. Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. J Natl Cancer Inst 1972; 49:891–5.
17. Takemura Y, Ohnuma T, Chou TC, Okano T, Holland JF. Biologic and pharmacologic effects of harringtonine on human leukemia-lymphoma cells. Cancer Chemother Pharmacol 1985; 14:206–10.
18. Kano Y, Ohnuma T, Holland JF. Folate requirements of methotrexate-resistant human acute lymphoblastic leukemia cell lines. Blood 1986; 68:586–91.
19. Diddens H, Niethammer D, Jackson RC. Patterns of cross-resistance to the antifolate drugs trimetrexate, metoprine, homofolate, and CB3717 in human lymphoma and osteosarcoma cells resistant to methotrexate. Cancer Res 1983; 43:5286–92.
20. Takemura Y, Ohnuma T, Miyachi H, Sekiguchi S. A human leukemia cell line made resistant to two folate analogues, trimetrexate and N^{10}-propargyl-5,8-dideazafolic acid (CB3717). J Cancer Res Clin Oncol 1991; 117:519–525.
21. Sikora E, Jackman AL, Newell DR, Calvert AH. Formation of retention and biological activity of N^{10}-propargyl-5,8-dideazafolic acid (CB3717) polyglutamates in L1210 cells in vitro. Biochem Pharmacol 1988; 37:4047–54.
22. Pawelczak K, Jones TR, Kempny M, et al. Quinazoline antifolates inhibiting thymidylate synthase: synthesis of four oligo (L-γ-glutamyl) conjugates of N^{10}-propargyl-5,8-dideazafolic acid and their enzyme inhibition. J Med Chem 1989; 32:160–5.
23. Cowan KH, Jolivet J. A methotrexate-resistant human breast cancer cell line with multiple defects, including diminished formation of methotrexate polyglutamates. J Biol Chem 1984; 259:10793–800.
24. Pizzorno G, Mini E, Coronnello M, et al. Impaired polyglutamylation of methotrexate as a cause of resistance in CCRF-CEM cells after short-term, high-dose treatment with this drug. Cancer Res 1988; 48:2149–55.
25. Jackman AL, Grezelakowska-Sztabert B, Newell DR, Calvert AH, Harrap KR. Transport studies with [^{3}H]-CB3717. Br J Cancer 1985; 52:431.
26. Jansen G, Schornagel JH, Westerhof GR, Rijksen G, Newell DR, Jackman AL. Multiple membrane transport systems for the uptake of folate-based thymidylate synthase inhibitors. Cancer Res 1990; 50:7544–8.

Submitted for publication December 1990.
Final revision received October 1991.

Peripancreatic Lymph Node Gastrinoma in a Patient with Zollinger-Ellison Syndrome

HIRSHEL KAHN, M.D., AND JENNY WAXMAN, M.D.

Abstract

The case of a patient who had Zollinger-Ellison syndrome caused by a primary peripancreatic lymph node gastrinoma is presented. Accompanying diffuse pancreatic islet cell hyperplasia, a well-documented occurrence in hypergastrinemia, was present. A perforated esophageal ulcer in a Barrett's esophagus led to right-sided necrotizing pleuritis, septicemia, and death. The diffuse parathyroid hyperplasia also noted in the patient is thought to be secondary to chronic hypertensive renal failure rather than MEN-I syndrome.

Case Report. A 75-year-old woman was admitted to the City Hospital Center at Elmhurst in April 1990 complaining of vomiting coffee-ground material of one day's duration. She had a history of Zollinger-Ellison syndrome diagnosed in 1976 based on positive results in secretin stimulation testing, a baseline serum gastrin level of 3969.0 ng/L (normal 0–100 ng/L), and the suggestion of a small lesion in the head of the pancreas on arteriography. This was complicated by esophageal strictures, and the patient had undergone multiple esophageal dilatations over several years. She was also being treated by hemodialysis for hypertensive chronic renal failure. She was not taking antihypertensive medication, but was being treated with Omeprazole, 40 mg a day. She was a nonsmoker and consumed alcohol occasionally.

Significant physical findings on admission included tachycardia, a fever of 99.8°F, and epigastric tenderness. Coffee-ground material was aspirated from the nasogastric tube.

Her white cell count was 15.7×10^9/L, hemoglobin 105 g/L, hematocrit .315, BUN 14 μmol/L (normal 3–6.5 μmol/L), and creatinine 470 μmol/L (normal 50–110 μmol/L). Calcium levels were within normal limits.

On the second day after admission, the patient suffered cardiorespiratory arrest and remained ventilator-dependent following resuscitation. A right pneumothorax was detected, and insertion of a chest tube drained 500 cc of brown, foul-smelling fluid. She was treated with intravenous antibiotics. She remained unresponsive and died later that day.

Pathologic Findings. At autopsy, the pancreas was firm and tan. Serial sectioning at 2- to 3-mm intervals revealed diffuse effacement of the parenchyma with loss of normal lobularity, but no discrete masses were identified. Microscopically, sections revealed interstitial fibrosis with ductal and ductular ectasia. Marked islet cell hyperplasia was noted in sections from the body and tail, where in some areas it replaced almost the entire exocrine component. Extensive sampling of the entire pancreas revealed no evidence of a microscopic gastrinoma.

Most remarkable, however, was the finding of two peripancreatic lymph nodes, anterior within the region of the pancreatic head, which

---

From the Lillian and Henry M. Stratton-Hans Popper Department of Pathology, Mount Sinai School of Medicine (CUNY), and the City Hospital Center at Elmhurst, New York. Address correspondence and reprint requests to Dr. Hirshel Kahn, Department of Pathology, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

were enlarged and firm, each measuring 1.0 × 0.8 cm. Sections revealed these to be almost entirely replaced by nests of regular, uniform tumor cells, with round speckled nuclei, and abundant granular eosinophilic cytoplasm (Fig. 1). Aggregates of lymphoid tissue at the rim of the tumor confirmed the gastrinoma to be within a lymph node. Immunohistochemical staining using the method described by Thung (1) revealed the tumor cells to be strongly positive for chromogranin and neuron-specific enolase and focally positive for gastrin (Fig. 2). Insulin staining was negative. Immunohistochemical staining of sections from the pancreatic islet cell hyperplasia showed a normal distribution of positivity for insulin, as well as positivity for neuron-specific enolase and chromogranin, but specimens were negative for gastrin (Fig. 3). These findings confirmed the presence of a gastrinoma in two peripancreatic lymph nodes.

The distal 12 cm of the esophagus showed superficial ulceration, with circumferential fibrosis and stricturing. A large (1.4 cm), well-circumscribed perforated ulcer was noted at a point 1.5 cm proximal to the gastroesophageal junction.

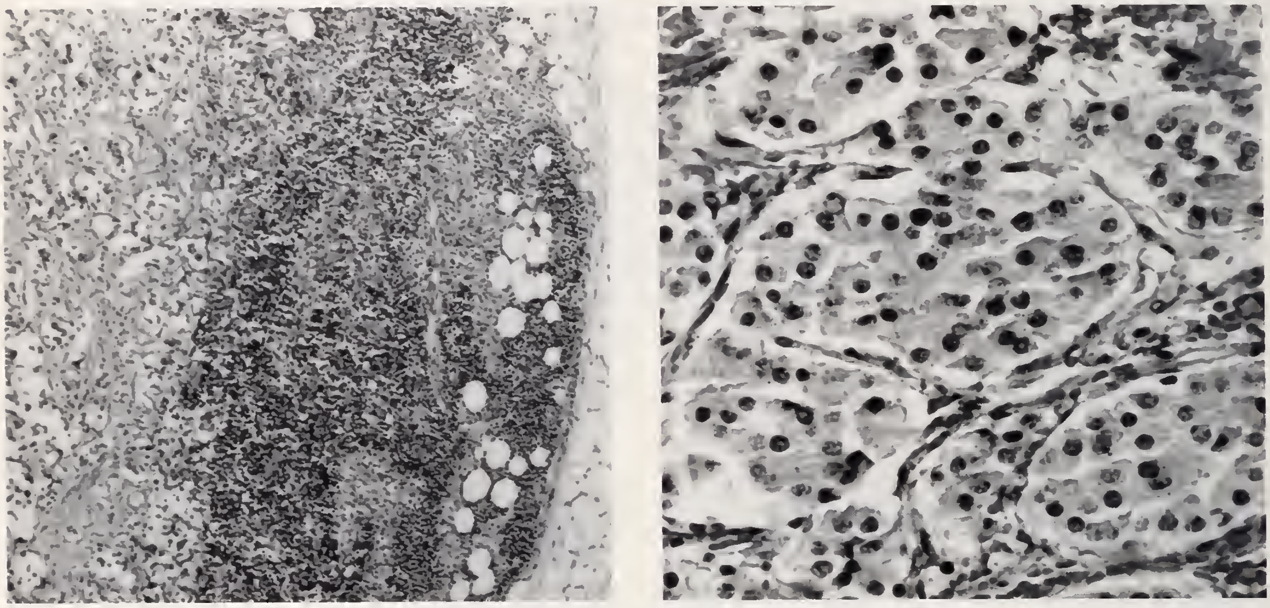
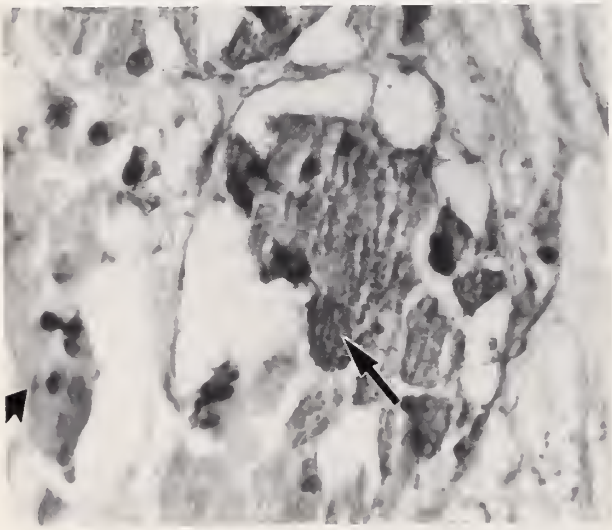


Fig. 1. Left Peripancreatic lymph node showing rim of cortical lymphocytes and nests of tumor cells, with postmortem autolysis. (Hematoxylin-eosin stain, original magnification ×40.) **Right** Nests of uniform tumor cells, with round, speckled nuclei and abundant granular eosinophilic cytoplasm. (Hematoxylin-eosin stain, original magnification ×200.)



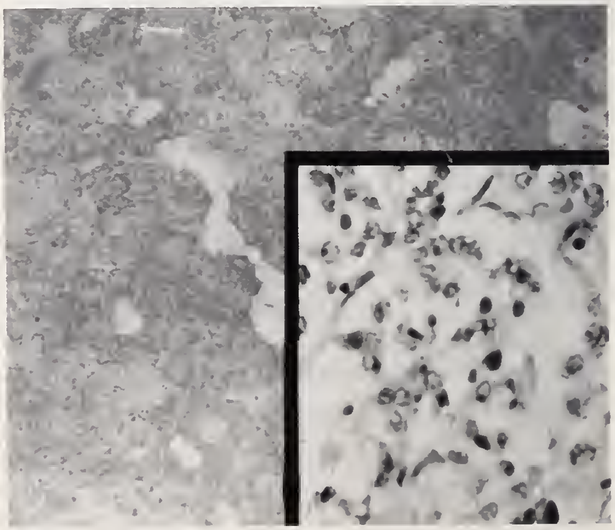


Fig. 2. Left Tumor nests in lymph node showing focal positivity for gastrin (long arrow), and negatively stained tumor (short arrow). (Immunoperoxidase stain, counterstained with Harris' hematoxylin, original magnification ×200.) **Fig. 3. Right** Pancreas showing diffuse islet cell hyperplasia, with negative gastrin staining. (Immunoperoxidase stain, counterstained with Harris' hematoxylin, original magnification ×20, inset ×200.)

Microscopic sections showed changes of chronic reflux esophagitis, gastric mucosal glands consistent with Barrett's esophagus, and transmural acute inflammation and necrosis at the site of perforation. The stomach showed slightly enlarged rugal folds on gross examination. Microscopic sections revealed marked parietal cell hyperplasia. Careful gross examination and multiple histologic sections of all organs, including stomach and duodenum, did not reveal evidence of gastrinoma in any organ other than the two peripancreatic lymph nodes.

The right pleural cavity revealed empyema, with acute hemorrhagic and necrotizing pleuritis. Cultures of blood and of right pleural fluid grew *Escherichia coli.*

The kidneys were markedly atrophic, weighing 35 g and 50 g. Sections revealed changes of end-stage renal disease, most consistent with a hypertensive etiology. No renal arterial stenosis was present.

All four parathyroid glands showed hyperplasia, predominantly oxyphilic. Bone showed changes of hyperparathyroidism, with resorption and paratrabecular fibrosis.

Discussion. The term Zollinger-Ellison syndrome has been used to describe acid hypersecretion and fulminant peptic ulcer disease in association with a non-insulin-producing tumor of the pancreas, most often gastrinoma (2, 3). In 90%–95% of patients, the gastrin-producing tumor is found within the pancreas (4). Extrapancreatic sites include the duodenum (the most frequent such site), wall of the stomach, hilus of spleen, omentum, mesentery, liver, ovary, and parathyroid. Gastrinomas in peripancreatic lymph nodes have also been reported, and it is not clear whether these foci have arisen in ectopic islet tissue or whether they represent metastases from undetected primary neoplasms (2, 5–7). Thirty percent of patients with gastrinomas have multiple endocrine neoplasia type I syndrome, parathyroid hyperplasia being the most commonly associated neoplasia (2). These patients usually have multiple small gastrinomas within the pancreas which are almost never resectable (8). At least 60% of gastrinomas are biologically malignant, and an estimated 50% are metastatic at the time of diagnosis (5, 7).

The case presented describes a patient with clinically diagnosed Zollinger-Ellison syndrome in whom, at autopsy, a gastrinoma was found in two peripancreatic lymph nodes. The question arises whether these were primary extrapancreatic gastrinomas or metastases from an occult intrapancreatic gastrinoma. Several reports of primary gastrinomas in peripancreatic lymph nodes are noted in the literature (2, 5, 7). The immediate return of the gastrin levels to normal after surgical resection of these tumors, and the sustained normal values after many years of follow-up, support the contention that these tumors are primary. Certain histologic features are more prominent in cases of primary nodal gastrinomas: the tumors are expansile rather than infiltrative, with formation of a fibrous pseudocapsule around the tumor; cystic degenerative changes; and the presence of hyalinized septa separating tumor nests. These histologic features were not apparent in our case, but no site of a primary tumor other than that in the lymph nodes could be found.

Islet cell hyperplasia is frequently associated with gastrin-producing tumors and other conditions producing hypergastrinemia (10). In approximately 10% of patients, hyperplasia of pancreatic endocrine tissue (islet cell hyperplasia) is described as a cause of Zollinger-Ellison syndrome (3). It seems likely, however, that cases of islet cell hyperplasia, previously thought to be the cause of Zollinger-Ellison syndrome, may in fact be secondary to an undetected microscopic gastrinoma. Our case demonstrated diffuse islet cell hyperplasia which stained negative for gastrin, suggesting that in this case it was probably an associated phenomenon. In a recent report of another almost identical case, it has been suggested that the peripancreatic tumor in the lymph nodes may in fact be a metastasis from microscopic nests of intrapancreatic gastrin-producing islet cells that had histologic features of islet cell hyperplasia (9). This situation has been compared to familial medullary carcinoma of the thyroid, in which transition from C-cell hyperplasia to microscopic medullary carcinoma is often subtle and in which metastatic disease may be seen with even minute lesions.

Whether the parathyroid hyperplasia of the patient reported on here was attributable to MEN-I syndrome or was secondary to her chronic renal failure cannot be answered with certainty. There was no family history of MEN. Permission for opening the skull was not granted; thus the pituitary gland was not examined. It was felt that the parathyroid hyperplasia was secondary to chronic renal failure, because serum calcium levels were within normal limits, and the bone changes of hyperparathyroidism (trabecular resorption by osteoclasts and paratrabecular fibrosis) were not as pronounced as would be expected in primary hyperparathyroidism.

Esophageal reflux and its complications have been well documented in Zollinger-Ellison syndrome (6). Reflux esophagitis may progress to superficial ulceration, circumferential fibrosis with stricture formation, and Barrett's esophagus. An ulceration may develop in this background, with resultant perforation, as noted in the case under discussion.

References

1. Thung SN. The development of proliferating ductular structures in liver disease. Arch Pathol Lab Med 1990; 114:407–411.
2. Howard TJ, Passaro E. Gastrinoma. New medical and surgical approaches. Surg Clin North Am 1989; 69(3):667–681.
3. Kloppel G, Heitz PU. Pancreatic pathology. New York: Churchill Livingstone, 1984.
4. McGuigan JE. Islet cell tumors and the ulcerogenic syndrome. In Fitzgerald PJ, Morrison AB (eds). The pancreas, International Academy of Pathology Monograph 21. Baltimore: Williams & Wilkins, 1980:177–184.
5. Bhagavan BS, Slavin RE, Goldberg J, et al. Ectopic gastronima and Zollinger-Ellison syndrome. Hum Pathol 1986; 17:584–592.
6. Rosai J. Ackerman's surgical pathology, 7th ed. St. Louis: CV Mosby, 1989.
7. Wolfe MM, Alexander RW, McGuigan JE. Extrapancreatic extraintestinal gastrinoma. N Engl J Med 1982; 306(25):1533–1536.
8. Deveney CW, Deveney KE. Zollinger-Ellison syndrome (gastrinoma). Surg Clin North Am 1987; 67(2):411–422.
9. Sawady J, Mendelsohn G. Extrapancreatic gastrinoma with pancreatic islet cell hyperplasia. Arch Pathol Lab Med 1989; 113:536–538.
10. Larsson L. Two distinct types of islet abnormalities associated with endocrine pancreatic tumors. Virchows Arch A Path 1977; 376:209–219.

Submitted for publication August 1991.
Final revision received December 1991.

Treatment of a Large High-Grade Neurofibrosarcoma with Concomitant Vinblastine, Doxorubicin, and Radiotherapy

Howard W. Bruckner, M.D., Mayer Gorbaty, M.D., Roberto Lipsztein, M.D., Giselle Kranjac, B.S., and Mark G. Lebwohl, M.D.

Abstract

A patient with neurofibromatosis developed a large inoperable malignant schwannoma on the posterior neck. The tumor underwent complete local regression following combined-modality treatment with radiotherapy, vinblastine, and doxorubicin. Vinblastine may be effective in combined-modality therapy.

NEUROFIBROSARCOMAS, also called malignant schwannomas, have been reported in 3.1% of patients with neurofibromatosis (1). Large foci of sarcomas present difficult management problems. Tumors often invade vital structures, resulting in pain, deep-seated infection, and loss of function. Current treatments usually produce inadequate benefit for patients with large sarcomas. This report describes a successful treatment for local disease with radiation (RT) and simultaneous chemotherapy consisting of doxorubicin (ADM) and vinblastine (VLB). Treatment produced a pathology-proven complete local disappearance of the tumor in the completely resected radiotherapy field.

Case Report. A 60-year-old man with neurofibromatosis was seen in July 1982. He was complaining of a profuse discharge from a painful, nonhealing ulceration that had been present on his posterior neck for several months. On examination, the patient had generalized cutaneous neurofibromatosis as evidenced by the presence of multiple soft skin-colored nodules and tumors covering most of his cutaneous surface. In addition, there was an indurated painful tumor, approximately 10 × 6 cm, on the left posterior neck (Fig. 1). The tumor was ulcerated with surrounding erythema and was not movable, appearing to be fixed to underlying structures. Resection in August 1982 was not successful, and histologic examination revealed multiple mitotic cells arising within a plexiform neurofibroma (Fig. 2). In October 1983, the tumor measured 12 × 11 × 6 cm and was fixed to underlying bone. The tumor was superinfected and diffusely tender. There was no evidence of metastatic disease.

Treatment consisted of doxorubicin, 50 mg/M^2, and vinblastine, 5 mg/M^2, administered on two occasions four weeks apart. On the first day of chemotherapy, radiotherapy was also begun and consisted of 4500 cGy in 23 fractions using parallel opposed lateral fields given over five weeks. The total dose of doxorubicin was 100 mg/M^2. The patient was hospitalized once without leukopenia for intravenous antibiotics because of fever to 103°F and purulent drainage from the necrotic tumor. No other adverse effects were noted. A wide resection with skin flap closure in January 1984 demonstrated a pathology-proven complete

---

From the Department of Neoplastic Diseases (HWB, MG), Department of Radiation Oncology (RL), and Department of Dermatology (GK, MGL), The Mount Sinai School of Medicine, New York, New York, 10029. Address reprint requests and correspondence to Mark G. Lebwohl, M.D., Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029.

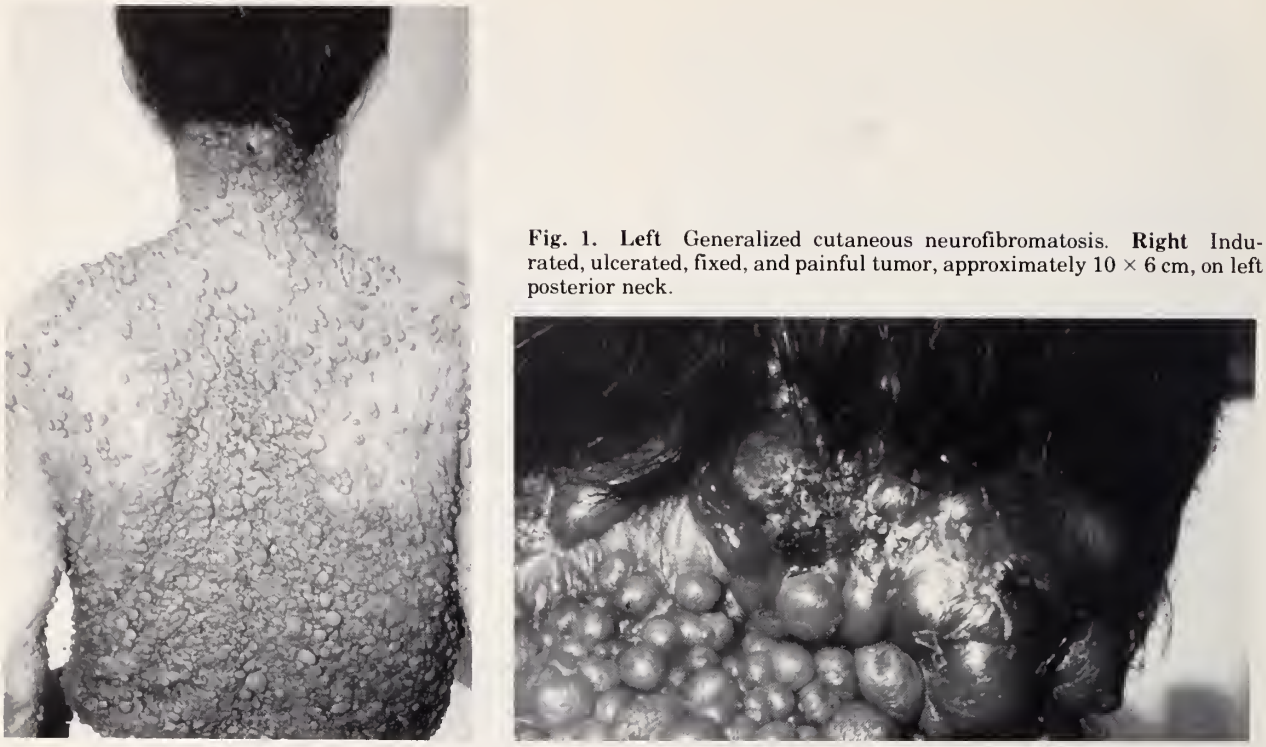


Fig. 1. **Left** Generalized cutaneous neurofibromatosis. **Right** Indurated, ulcerated, fixed, and painful tumor, approximately 10 × 6 cm, on left posterior neck.

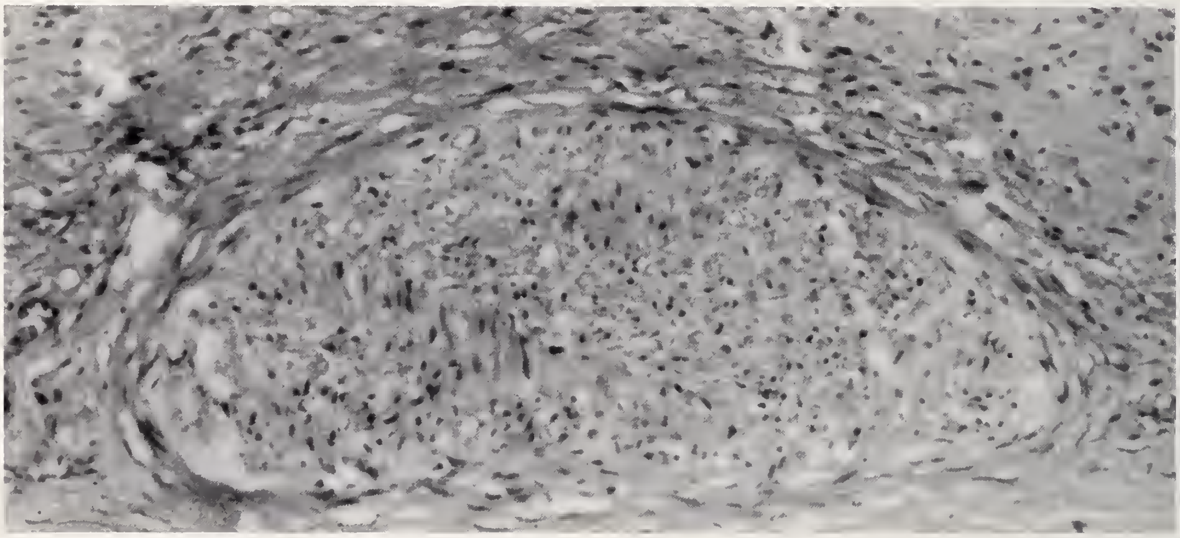


Fig. 2. Histologic examination of tumor revealed multiple mitotic cells arising within plexiform neurofibroma.

remission. Maintenance chemotherapy was not given. Five months later, the patient developed bilateral pulmonary metastases without evidence of a local recurrence. Shortly thereafter, the patient died of pulmonary edema before further therapy could be given.

Comment

Neither drugs nor radiation has been reported to produce complete remission of an undifferentiated malignant schwannoma. This tumor is among the most refractory to treatment.

Complete local response in this patient cannot be attributed to the modest doses of radiation administered. In doses of 4500–5500 cGy, radiation is only sometimes effective in controlling the microscopic residual of a sarcoma. Gross disease requires substantially larger doses, in the range of 7000 cGy or higher, and the quality of the control is often poor (2).

Doxorubicin produces a complete response rate of only 10%, and the complete responses usually are observed in initially small sarcomas (3). Treatment with RT and doxorubicin has not produced complete remission of large sarcomas. The observed progression of distant disease eliminates the possibility that these complete remissions of large tumors were due to the chemotherapy alone. Doxorubicin has been used with radiation in the treatment of several tumors and appears to heighten the response of normal tissues (4). It produces in vitro synergy, secondary to interference with the repair of radiation-induced DNA damage (5).

Vinblastine produces a response rate of less than 5% against common sarcomas and has never produced complete response in large sarcomas (1, 6). Vinblastine has been identified as a candidate drug for treatment (testing) against some rare "sarcomas": adjuvant therapy of angiosarcoma of the breast (7), rhabdosarcoma of the urinary bladder (8), and ocular reticulum cell "sarcoma" (9). Some preclinical information identifies desacetyl vinblastine amide as a possible treatment for lipoblastic liposarcoma and malignant schwannoma (10). Combination chemotherapy employing other alkaloids may infrequently produce complete responses: 1 out of 14 (11); 6 out of 31 (12); 2 out of 23 (13). These complete responses usually occur in small tumors.

To our knowledge, vinblastine has been only minimally tested as a radiation sensitizer. Preliminary preclinical evidence suggests that velban has a radiosensitizing effect (14, 15) and that scheduling of RT-VLB can be critical to the efficacy of velban (15–17). The combination of doxorubicin-vinblastine may be either additive or synergistic (18).

Vinblastine is one of several alkaloids effective against a wide spectrum of disease. It can be synergistic with other radiosensitizers such as cisplatin. The recent successes of combined-modality therapy with halogenated pyrimidines with and without cisplatin encourage evaluation of additional drugs such as vinblastine for similar applications. Initial experience of others (13 of 26 patients with apparent benefit) encourages testing of alkaloids in a comprehensive multidisciplinary strategy to improve the results of radiotherapy and surgery (10, 12). This treatment has also produced complete remission of a large uterine sarcoma that obliterated the apex of the lung, fixed the scapula, and, by direct extension, produced a palpable mass (unpublished data).

Conclusions and Summary. It should be stressed that only the local tumor that was particularly problematic was successfully treated with this regimen. However, the regimen was not curative, and the patient did develop pulmonary metastases after a five-month remission. Unfortunately, the patient died of pulmonary edema before further treatment could be instituted. Nevertheless, the temporary dramatic benefit obtained in this patient warrants use of this regimen in other patients with neurofibrosarcomas that fail to respond to other therapeutic modalities.

References

1. D'Agostino AN, Soule EH, Miller RH. Sarcomas of the peripheral nerves and somatic soft tissues associated with multiple neurofibromatosis (von Recklinghausen's disease). Cancer 1963; 16:1015–1027.
2. Rosenberg SA, Suit HD, Baker LH, Rosen G. Sarcomas of the soft tissue and bone. In: Devita VT, Hellman S, Rosenberg SA, eds. Cancer principles and practice of oncology. Philadelphia: JB Lippincott, 1982:1036–1093.
3. Blum RH, Carter SK. Adriamycin. A new anticancer drug with significant clinical activity. Ann Intern Med 1974; 80:249–259.
4. Horwich A, Lokich JJ, Bloomer WD. Doxorubicin, radiotherapy and oesophageal stricture. Lancet 1975; 2:561–562.
5. Watring WG, Byfield JE, Lagasse LN, et al. Combination adriamycin and radiation therapy in gynecologic cancers. Gynecol Oncol 1974; 2:518–526.
6. Selawry OS, Holland JF, Wolman IJ. Effect of vincristine (NSC 67574) on malignant solid tumor in children. Cancer Chemother Rep 1968; 52:497–499.
7. Rosner D. Primary angiosarcoma of the breast: possible cure by adjuvant chemotherapy. Proc Am Soc Clin Oncol 1983; 2:C-882.
8. Tawa A, Yabuta R, Yuumura K, et al. Rhabdomyosarcoma of the urinary bladder: complete remission induced by vinblastine, cisplatinum, and bleomycin. Gan To Kagaku Ryoho 1982; 9(12):2222–2228.
9. Char DH, Margolis L, Newman AB. Ocular reticulum cell sarcoma. Am J Ophthalmol 1981; 91(4):480–483.
10. Sordillo PP, Hajdu SI, Magill GB, Lesser M, Helson L. Effect of desacetyl vinblastine amide (DVA) against human sarcomas heterotransplanted in nude mice. Cancer Clin Trials 1980; 3(4):391–394.
11. Rouesse J, Le Chevalier T, Contesso G, et al. Primary chemotherapy in soft tissue sarcomas considered inoperable. Sem Hop Paris 1983; 59(19):1441–1444.
12. Brenner J, Magill GB, Wissel P, Sordillo PP. Chemotherapy of patients with advanced soft tissue sarcoma with use of DVA (vindesine sulfate), adriamycin and cyclophosphamide (DAC). Cancer 1983; 52(7):1142–1145.
13. Maree D, Hocke C, Bui NB, et al. Conservative locoregional treatment of soft tissue sarcoma in adults after induction chemotherapy. Presse Med 1985; 14(19): 1069–1072.
14. Storme G, De Neve W, Mareel M. Vinblastine, a microtubule inhibitor, has a radiosensitizing effect. Proc Am Assoc Cancer Res 1985; 26:237.

15. Todoroki T, Koite S, Tsunemoto H, Watanabe I. Enhanced radiation lethality in partially synchronized solid mouse tumors. Cancer Res 1982; 42(12):5231–5235.
16. Barendsen GW. Experimental studies on combined radiochemotherapy. Bull Cancer (Paris) 1981; 68(2):121–126.
17. Barendsen GW, Janse HC. Differences in effectiveness of combined treatments with ionizing radiation and vinblastine, evaluated for experimental sarcomas and squamous cell carcinomas in rats. Int J Radiat Oncol Biol Phys 1978; 4:95–102.
18. Perloff M, Hart RD, Holland JF. Vinblastine, adriamycin, thioTEPA and halotestin (VATH) therapy for advanced breast cancer refractory to prior chemotherapy. Cancer 1978; 42:2534–2537.

Submitted for publication July 1991.
Final revision received March 1992.

Serotonin Antagonists:

Treatment of Chemotherapy-Induced Emesis

Anthony Barisano, Pharm. D., Bernard Mehl, D.P.S., and Keith Bradbury, M.S.

In mid-1991 the Mount Sinai Pharmacy and Therapeutics Committee considered the new antiemetic agent ondansetron for addition to the Formulary because of its promise of improved efficacy and fewer side effects in treating chemotherapy-induced nausea and vomiting. Chemotherapy-induced emesis (CIE) remains one of the most significant adverse effects of cancer treatment. Although considerable progress has been made in recent years in the prevention of CIE, it remains a critical problem in cancer treatment. Prolonged episodes of nausea and vomiting may result in malnutrition, weight loss, electrolyte imbalance, dehydration, and esophageal tears. In addition, most patients experience general malaise and decreased energy, leading to inability to perform daily activities, which in turn yields deterioration in their quality of life. Emesis may be severe enough to lead to delay or termination of subsequent, and potentially curative, chemotherapy regimens.

Prior to the mid-1970s, phenothiazines constituted the mainstay of treatment. Subsequently, several newer classes of antiemetics were identified: butyrophenones, cannabinoids, substituted benzamides, and corticosteroids. Despite numerous advances in the control of CIE, significant problems remain. It is estimated that one-third of patients receiving cisplatin or a cisplatin-containing regimen still experience emesis within the first 24 hr after treatment (1).

Complete control of anticipatory and delayed emesis is still unaccomplished. An alternative effective, nontoxic antiemetic agent is still needed. The table lists the emetogenic potential of various chemotherapeutic agents.

Research over the past five years has resulted in the development of a new class of antiemetic agents known as serotonin antagonists. The physiology of acute drug- and radiation-induced emesis has been reevaluated with this new understanding of the possible role of serotonin. There are four different subtypes of serotonin receptors ($5HT_1$–$5HT_4$). The most relevant to antiemesis are the $5HT_3$ receptors found in the enterocromaffin cells (EC) of the gastroesophageal junction, duodenum, and in the area postrema where the chemoreceptor trigger zone (CTZ) resides. More than 80% of the total body content of serotonin is located in the EC of the mucosal layer (2). It is theorized that antineoplastic agents cause emesis either by direct stimulation of $5HT_3$ receptors in the CTZ or by causing the release of serotonin from EC in the gut wall. This release will activate the $5HT_3$ receptors in the gastrointestinal vagal nerves, yielding a direct stimulus of the vomiting center and CTZ.

Numerous serotonin antagonists have been developed. As a class they possess a high degree of specificity for binding to $5HT_3$ receptors. Several have undergone clinical testing: ondansetron (Zofran, Glaxo), granisetron (BRL-43694A, Smith Kline Beecham), zacopride (AHR-1190, Robins), batanopride (BMY-25801, Bristol), MDL-73147 (Merrell Dow), RG-12915 (Rhone-Poulenc-Rorer), and tropisetron (ICS-205930). Only ondanestron is FDA approved. The others remain investigational; however, approval of granisetron is anticipated.

Agents Clinically Tested

Ondansetron. Ondansetron, approved by the Food and Drug Administration in March 1991, appears to be effective as prophylactic therapy for

---

From the Department of Pharmacy, Mount Sinai Medical Center. Address reprint requests to Bernard Mehl, D.P.S., Director, Dept. of Pharmacy, Box 1211, The Mount Sinai Hospital, One Gustave L. Levy Place, New York, NY 10029.

the prevention of cisplatin-induced emesis. Several studies have shown that a daily dose of 32 mg, given either as a continuous infusion (CI) (8-mg bolus followed by a 1-mg/hr CI) or intermittently (8-mg bolus followed by 8 mg every 8 hr) can provide effective control of emesis (3). Studies in which ondansetron was administered for three doses given at intervals of 2, 4, 6, and 8 hr have all shown comparable antiemetic efficacy (4a, b). Two studies addressing the question of late-onset emesis showed no improvement in emesis control with a total of six doses of 0.18 mg/kg over the three doses used in prior studies (5a, b). Ondansetron has also proved effective in radiation-induced emesis. Following a loading and an every-8-hr dose, 91% of children (aged 2–17 years) had fewer than five emetic events following total body irradiation; 55% had no vomiting at all (6). Ondansetron has been studied to a limited extent in combination with glucocorticoids and results suggest additive antiemetic efficacy (7).

The recommended dose is 0.15 mg/kg infused over 15 min every four hr for three doses. The first dose is infused 30 min prior to chemotherapy. Ondansetron has a relatively low toxicity profile. The most common side effects include headache (the only dose-related side effect), constipation (probably due to $5HT_3$ blockade in the colon), transaminase elevations (up to two times normal), and mild sedation. Ondansetron does not have any dopamine receptor antagonism activity; therefore it is free of the extrapyramidal side effects.

Granisetron. Granisetron is an extremely potent serotonin antagonist and was the first serotonin antagonist shown to abolish radiation-induced emesis in an animal model (8). Clinical trials in cancer patients showed that, compared with placebo, granisetron significantly reduces the incidence of nausea and vomiting for 24 hr after administration of high-dose cisplatin (>49 mg/m^2) (9). In a large multicenter trial, single-dose (40 μg/kg) granisetron was compared to high-dose metoclopramide and dexamethasone in patients receiving high-dose cisplatin. Both groups obtained complete control of emesis in 70% of patients (10). However, granisetron proved superior to a combination of chlorpromazine plus dexamethasone, or prochlorperazine plus dexamethasone, or methylprednisolone monotherapy (11).

The pharmacokinetic values pertain to the parenteral dosage form. Granisetron exhibits linear kinetics over a wide dosage range (10–300 μg/kg). The mean plasma half-life is longer in cancer patients (10–12 hr) than in healthy volunteers (3.1–5.9 hr). The elimination of granisetron is primarily by nonrenal mechanisms, with only 8%–15% of the parent compound recovered in the urine. The most frequently reported adverse effect is headache, which occurs in 10%–15% of patients; constipation, somnolence, diarrhea, and transient changes in blood pressure have been reported less frequently. In most clinical trials, single IV doses of 40–160 μg/kg were administered five minutes before chemotherapy. Recently initiated multicenter double-blind trials involving Mount Sinai Medical Center randomize patients to the FDA dosage of ondansetron or 40-60 mg of granisetron with high-dose cisplatin therapy.

TABLE

Emetogenic Potential of Chemotherapy Agents

High*	Moderate	Mild
Cisplatinum	Carmustine	Etoposide
Dacarbazine	Lomustine	Mitomycin
Dactinomycin	Anthracyclines < 40 mg/m^2	Methotrexate
Mechlorethamine	Cytarabine < 500 mg/m^2	Fluorouracil
Cyclophosphamide	Carboplatin	Hydroxyurea
Ifosfamide	Etoposide > 100 mg/m^2	Bleomycin
Streptozocin	Vincristine	Mitoxantrone
Cytarabine > 500 mg/m^2	Vinblastine	
Anthracyclines > 40 mg/m^2		

* Adapted from Devita et al. (22).

Zacopride. Zacopride is a substituted benzamide with extremely potent serotonin antagonism. Preclinical activity is shown in the nanogram dose range (10–1000 ng). This agent, however, has not been particularly effective in control of emesis (12) because it induces emesis through its gastroprokinetic properties, which overlap its serotonin antagonism (13, 14).

Batanopride. Batanopride, also a substituted benzamide, has a greater affinity for the serotonin receptor (fourfold) than does metoclopramide and is inactive against dopamine-induced emesis. Batanopride has been shown to be effective in controlling CIE at doses of 1.2–8 mg/kg administered IV every four hours for three doses (15, 16). In a recent placebo-controlled trial, patients with head and neck cancers received cisplatin 100 mg/m^2. Batanopride gave complete control of acute emesis in 12 of 15 patients versus 2 of 14 placebo-treated patients (17). Adverse events experienced include dose-related and dose-limited transient lengthening of the QT interval on electrocardiogram and severe but reversible hypotension; no extrapyramidal symptoms were noted.

MDL-73147. MDL-73147, a new compound, has undergone minimal clinical testing. The antiemetic efficacy was recently reported in a phase 1 study where antiemetic protection (more than two episodes of emesis) was observed in 80% of patients. A dose range of 10–50 mg was administered and no differences in side effects or efficacy were noted. The main side effect was mild headache (18).

RG-12915. RG-12915, a substituted carboxamide, has good oral bioavailability, unlike ondansetron, and has been shown to be effective, in the ferret and dog model, for inhibiting cisplatin-induced emesis (19). It possesses an elimination half-life of approximately 8.7 hr. Twelve of 12 patients, in a phase 1 study, experienced discomfort at the infusion site. RG-12915 is currently undergoing a placebo-controlled, double-blind study, at The Mount Sinai Medical Center, New York City, for the treatment of postoperative nausea.

Tropisetron. Tropisetron is a potent serotonin receptor antagonist. Pharmacokinetic data demonstrate a long terminal half-life of approximately 11 hr. However, there has been minimal clinical testing to date with tropisetron. In phase 2 and 3 studies, tropisetron has been administered in doses ranging from 5 to 40 mg as a single IV infusion. All patients were receiving high-dose (>50 mg/m^2) cisplatin; in these studies, total control of emesis, ranged from 54% to 66% (20, 21). The authors concluded, although antiemetic activity was observed, that overall efficacy was less than anticipated. Direct comparisons with other serotonin antagonists are needed for final determinations. Headache and constipation comprised the most frequent adverse events noted.

In summary, it appears that the serotonin antagonists are an effective and safe group of agents for the prevention of acute emesis due to chemotherapy. They are not, however, the final solution to the problem. In addition to demonstrating efficacy with cisplatin and non-cisplatin-based therapy, serotonin antagonists exhibit no dopaminergic antagonism and therefore are free of extrapyramidal side effects. The serotonin antagonists are, however, very expensive, and their overuse in situations where metoclopramide may be just as effective is an issue that must be addressed. Furthermore, the data supporting efficacy for delayed emesis and/or multiple-day chemotherapy-related emesis is less than encouraging. Future directions will likely yield direct comparisons of serotonin antagonists and combinations with other antiemetic agents bearing alternative mechanisms of action.

Guidelines for Use of Ondansetron

The Mount Sinai Formulary Committee was challenged to develop a mechanism making ondansetron available for those inpatients who require it while minimizing the additional cost to the hospital, which was expected to be over $500,000 per year. The ondansetron charge to the patient is approximately $140 for a three-dose regimen, compared to $42–$70 for a traditional antiemetic combination regimen of three or four drugs. If ondansetron is used in combination with other agents, for instance lorazepam, the difference in charge is even greater ($182 vs. $42–$70). To help avoid unwarranted use of new drug products with significant financial implications, a subcommittee of oncologists, hematologists, and pharmacists was formed to prepare prescribing guidelines for the appropriate use of ondansetron. The following guide was the result.

1. Patients who have experienced little or no nausea and vomiting or no significant side effects when treated with traditional antiemetic combination therapy should continue to receive these agents. Ondansetron should not be prescribed in this setting.
2. Ondansetron may be used as a three-dose regimen (one before and two after) of 0.15 mg/kg for the prevention of acute (first 24 hr) chemotherapy-induced nausea and vomiting in the following patients:
   A. Infants and children, who are known to experience a high incidence of extrapyramidal side effects from dopamine blocking agents.
   B. Elderly patients over the age of 60, who are more prone to sedation from traditional antiemetic regimens.
   C. While an initial trial of combination antiemetic therapy with metoclopramide, diphenhydramine, lorazepam, and dexamethasone can be considered, patients receiving highly emetogenic chemotherapy agents can receive ondansetron.
   D. Patients receiving chemotherapy regimens that contain mildly or moderately emetogenic agents and who have experienced nausea and vomiting when treated with other combination regimens can receive ondansetron.
3. Ondansetron should not be used for the treatment of delayed chemotherapy-induced nausea and vomiting unless the patient fails to respond to other antiemetic agents.

Since a unique advantage of ondansetron is that it does not cause sedation or akathesia, other medications with sedative properties, e.g., lorazepam, should not be routinely needed as part of the antiemetic regimen. Lorazepam could be used when sedation and amnesia are desired.

These guidelines were subsequently mailed to all oncologists and hematologists who prescribe chemotherapy. The utilization of this drug has been subsequently reviewed on an ongoing basis to determine compliance with the guidelines and to assess the need for revisions. Through this process, the use of the drug is closely followed, and prescribers are consulted if the drug is used in a manner different from that recommended by the Committee.

As anticipated, use of this new agent increased rapidly, and the initial estimates of over $500,000/year expenditures for this drug proved to be conservative. In general, based on aggregate data from the Mount Sinai Medical Records Department, this has not been associated with a significant shortening of hospitalization, as was suggested by some clinicians, although individual patients may have shorter stays.

Based on data collected thus far, the use of this agent has been consistent with the guidelines, except in a few cases where the drug was used for nausea and vomiting secondary to amphotericin treatment, or in AIDS patients with intractable nausea and vomiting, and in several instances in which more than three doses were administered along with chemotherapy. In addition, in certain settings, e.g., radiation-induced nausea and vomiting in bone marrow transplant patients, ondansetron was being administered every four hours around the clock. This has been changed to administration every eight hours in these patients, based on the drug's pharmacokinetics and results reported in the literature (3). This change alone could lower cost to the institution for this agent by $50,000.

It is anticipated that the process of keeping prescribers informed regarding the findings of our review and the costs associated with new drugs will produce a cost benefit and promote cost-effective prescribing in this era of decreasing reimbursement and increased drug costs. Ondansetron is the first of a novel class of agents that possess a high degree of specificity for binding to $5HT_3$ receptors. This new class of agents and the promise that they offer in coping with the significant problem of chemotherapy-induced nausea and vomiting will continue to challenge pharmacy and therapeutics committees with selecting the most efficacious and economical therapies.

References

1. Kaasa S, et al. A comparison of ondansetron with metoclopramide in the prophylaxis of chemotherapy-induced nausea and vomiting: a randomized, double blind study. Eur J Cancer Clin Oncol 1990; 26:311–314.
2. Resnick RH, et al. Distribution of serotonin in the human gastrointestinal tract. Gastroenterology 1961; 41:119–121.
3. Marty M, et al. Comparison of the serotonin antagonist ondansetron with high-dose metoclopramide in the control of cisplatin-induced emesis. N Engl J Med 1990; 3222:816–821.

4a. Kris MG, et al. Phase 2 trials of the serotonin antagonist GR38032f for the control of vomiting caused by cisplatin. J Natl Cancer Inst 1989; 81:42–46.

4b. Hesketh PJ, et al. GR3802f: a novel compound effective in the prevention of acute cisplatin-induced emesis. J Clin Oncol 1989; 7:700–705.

5a. Sartiano G, et al. GR38023f: a new antiemetic effective in the prevention of cisplatin-induced nausea and vomiting. Proc Am Soc Clin Oncol 1989; 8:326.

5b. Hesketh PJ, et al. An open label dose comparison of intravenous GR38032f in the prevention of emesis induced by high dose cisplatin. Proc Am Soc Clin Oncol 1989; 8:333.

6. Hewitt M, et al. Effective antiemetic control during conditioning of children for bone marrow transplantation using ondansetron, a serotonin antagonist. Bone Marrow Transplant 1991; 7:431–433.
7. Roila F, et al. Prevention of cisplatin-induced emesis: a double blind multicenter randomized crossover study comparing ondansetron and ondansetron plus dexamethasone. J Clin Oncol 1991; 9:675–678.
8. Bermudez J, et al. The antiemetic potential of the serotonin receptor antagonist BRL-43694. Br J Cancer 1988; 58:644–650.
9. Cupissol DR, et al. The efficacy of granisetron as a prophylactic antiemetic and intervention agent in high-dose cisplatin-induced emesis. Eur J Cancer 1990; 26:s23–s27.
10. Chevallier B. Efficacy and safety of granisetron compared with high-dose metoclopramide plus dexamethasone in patients receiving high-dose cisplatin in a single blind study. Eur J Cancer 1990; 26:s33–s36.
11. Marty M, et al. A comparative study of the use of granisetron, a selective serotonin antagonist, versus a standard antiemetic regimen of chlorpromazine plus dexamethasone in the treatment of cytostatic-induced emesis. Eur J Cancer 1990; 26(Suppl 1):s28–s32.
12. Perez EA, et al. Exploratory dose-finding study of zacopride in the prevention of cisplatin-induced emesis. Proc Am Soc Clin Oncol 1990; 9:337.
13. Smith WW, et al. Zacopride, a potent serotonin antagonist. J Pharm Pharmacol 1988; 40:301–302.
14. Smith WW, et al. The antiemetic profile of zacopride. J Pharm Pharmacol 1989; 41:101–105.
15. Aapro MS, et al. BMY25801-01: a novel antiemetic to prevent cisplatin-induced nausea and vomiting. Proc Am Soc Clin Oncol 1987; 6:272.
16. Smaldone, et al. Dose range evaluation of BMY-25801, a non-dopaminergic antiemetic. Proc Am Soc Clin Oncol 1988; 7:280.
17. Bleiberg H, et al. Randomized double blind crossover eval-

uation of batanopride (BMY-25801) vs placebo in patients receiving high-dose cisplatin. Proc Am Soc Clin Oncol 1990; 9:338.

18. Boyce MJ, et al. Early clinical studies with MDL-73147ef, a new and selective serotonin receptor antagonist. Perugia, Italy: Perugia International Conference III, June 1990, p 89.
19. Fitzpatrick LR, et al. A potent serotonin antagonist that is an orally effective inhibitor of cytotoxic drug-induced emesis in the ferret and dog. J Pharmacol Exp Ther 1990; 254:450–455.
20. Stamatakis L, et al. ICS 205-930: a dose finding study in the prevention of cisplatin-induced nausea and vomiting. Proc Am Soc Clin Oncol 1989; 8:327.
21. Leibundgt U, et al. ICS 205-930: a serotonin receptor antagonist to prevent cisplatin-induced emesis. Proc Am Soc Clin Oncol 1988; 7:284.
22. Devita VT, Hellman S, Rosenberg SA. Cancer principles and practice of oncology, 3rd ed. 2138.

Submitted for publication March 1992.

Critical Care America is Sensitive to Your Special Needs

Critical Care America (CCA), an innovative leader in home infusion therapy, is dedicated to providing superior quality care and support to oncology patients in the home setting.

CCA offers a wide range of both traditional and innovative home infusion therapies, and complements them with 24 hour expert nursing, pharmacy, materials management and reimbursement services.

We are sensitive to the special needs of the oncology patient, and have developed a specialized program to help meet their needs and the needs of the physician more effectively and efficiently than any other home care provider.

Please call us toll-free for more information.
(800) 722-7003 / (800) 344-5500

Critical Care
AMERICA

When you don t know all the medicines your patients are taking, protecting their health is a crap shoot.

Counseling patients about all their medicines improves their odds of getting well and staying well.

EVERYONE WINS WHEN YOU TALK

Send me a *free* Medicine Counseling Kit.

Name

Organization

Street

City State Zip

Mail to:
National Council on Patient Information and Education
666 Eleventh Street, NW, Suite 810
Washington, DC 20001
To fax your request -- (202) 638-0773

this publication is available in microform

Please send me additional information.

University Microfilms International

300 North Zeeb Road
Dept. P.R.
Ann Arbor, MI 48106
U.S.A.

18 Bedford Row
Dept. P.R.
London, WC1R 4EJ
England

Name

Institution

Street

City

State Zip

Instructions for Authors

THE MOUNT SINAI JOURNAL OF MEDICINE, established in 1934 as *The Journal of the Mount Sinai Hospital,* is a peer-reviewed general medical journal. It is indexed or abstracted in *Index Medicus, Current Contents, Science Citation Index, Hospital Literature Index, International Nursing Index, Excerpta Medica, Chemical Abstracts, Biological Abstracts,* and major databases. Theoretical and basic science articles for the clinician, reviews, reports of clinical or research experience, articles on any subject affecting the practice of medicine, short notes in any specialty, book reviews, letters, and articles by medical students are welcome. The *Journal* is a participant in the Agreement on Uniform Requirements for Manuscripts Submitted to Biomedical Journals.

Communications. Address correspondence on manuscripts review to: Managing Editor, *The Mount Sinai Journal of Medicine,* Box 1094, 19 E. 98th Street, 4D, New York, NY 10029-6574. Phone: (212) 241-6108; FAX: (212) 722-6386.

Copyright. The publisher reserves copyright and renewal rights on all published material, and such material may not be published elsewhere without written permission of the publisher. When submitting a manuscript, include the following assignment of copyright in your cover letter: **In consideration of The Mount Sinai Journal of Medicine (MSJM) taking action and reviewing and editing my (our) submission, the author(s) undersigned hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership to The Mount Sinai Journal of Medicine in the event that such work is published by MSJM. Each author must sign the statement.**

Manuscript Preparation. Submit manuscripts in triplicate, including three sets of illustrations. Manuscripts must be typewritten, **double-spaced throughout (including references, legends, and tables)**, on one side of 8½ × 11-in. white bond paper, with generous margins. Number pages consecutively. Begin a new page for title page, abstract, first page of text, references, each table, and legends. **Computer-generated typescripts** must be double-spaced and must be easy for editors and typesetters to read. Computer tapes or disks: consult managing editor.

Title Page. On the title page, on separate lines (double-spaced), include (1) title of article, phrased as concisely as possible; (2) name of each author, including first name and degree; (3) name and address of departments and institutions from which the work originated; (4) acknowledgments of sources of support; (5) name, place, and date of any conferences at which paper has been delivered; (6) the statement Address reprint requests to . . . , giving full name and address, with zip code, of the appropriate author.

Abstract. Original articles should include an abstract, which should be a single paragraph not exceeding 150 words. State the purposes; basic approach or methods; main findings; principal conclusions. Emphasize new and important aspects. When an abstract is provided, a summary at the end is not necessary. Reviews and other articles without an abstract should conclude with a short summary.

Key Words. Supply 3 to 10 key words or phrases at the bottom of the title page, for use in indexing the article. Use Medical Subject Headings from *Index Medicus.*

Writing Style and Abbreviations. Authors should aim for conciseness and clarity without repetition. Avoid medical jargon, abbreviated phrasing, and obscure or newly coined abbreviations. Define all abbreviations on first use, except those for standard, universally recognized units of measurement and statistical terms. Spell out such terms as white blood cell, hematocrit, superior vena cava.

Units of Measurement. Use SI units, giving range or interval of normal values.

Editing. All papers will be edited to enhance conciseness and clarity without altering meaning and to insure conformity with journal style. The author is responsible for all statements in the article.

Illustrations. Photographs, line drawings, graphs, and charts should increase understanding of the text. Line drawings, graphs, and charts should be professionally prepared. All illustrations should be submitted in triplicate as sharp, high-contrast glossy prints. Photomicrographs must be 5⅛ in. wide. On the back of each print affix a gum label with the number of the figure (numbered consecutively in arabic numerals as cited in text), title of manuscript, name of senior author, and the word "top" with an arrow indicating top edge. Illustrations which are to appear together should be mounted accordingly as plates and should correspond to each other in size. Protect the prints by enclosing them in heavy cardboard; do not use paper clips. Photographs of patients can be published only if a copy of the permission is submitted with the photograph. Eliminate names of patients and hospital numbers from x-rays.

Legends. Legends should not duplicate the text. Double space. Number each legend to match the illustration it accompanies. Illustrations mounted as plates to appear together should be accompanied by a single legend identifying the separate elements by position ("upper left," and so on) or by letters. If letters are used in the legend, a corresponding letter must appear on the print itself. Identify all abbreviations. Indicate magnification and stain for photomicrographs.

Tables. Each table should be typed on a separate page, double spaced. Number tables consecutively as they appear in text, using arabic numerals ("Table 1"). Give each table a brief title (for example, "Effect of DMSO on Rats") and type it on a separate line. If abbreviations or symbols are used in the table, identify them in a footnote.

References. When citing references in your text, the first work cited is numbered 1, and succeeding references are numbered in sequence; enclose the citation number in parentheses and place it before any punctuation: **Cytomegalovirus (1), steroids (2), and recreational drugs (3) have all been implicated (4).** The reference list includes only works cited in the text, numbered in the order in which they are cited. Type, double spaced, following the general arrangement and punctuation style in the examples below (journal title abbreviations conform to *Index Medicus* style):

1. Nadelman RB, Wormser GP. A clinical approach to Lyme disease. Mt Sinai J Med 1990;57:144–156.

2. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. N Engl J Med 1991;324:424–428.

When a manuscript is accepted for publication, you will be asked to confirm the accuracy of all references.

Proofs. Proofs are sent to the corresponding author from the printer. Stylistic changes which do not alter meaning should not be made at this stage. Because of increased printing charges, the cost of excessive author's alterations beyond routine correction of typographical errors and major errors in data may be billed to the author. Proofs should be corrected and returned to the editorial office within 48 hours.

Reprint Orders. A reprint order form is sent to the corresponding author with proofs; return it to the editorial office.

THE
MOUNT SINAI JOURNAL
OF MEDICINE

Forthcoming:

Grand Rounds

Narrative and Medicine
Oliver Sacks
Albert Einstein College of Medicine

Osteoporosis: On the Verge of Rational Effective Therapy?
John Paul Bilezikian
Columbia University College of Physicians and Surgeons

The Global Impact of Penicillin: Then and Now
Gene H. Stollerman
Boston University School of Medicine

Obscure Gastrointestinal Bleeding
Blair Lewis
Mount Sinai School of Medicine

Pharmacology of Antiinflammatory Agents: A New Paradigm
Bruce N. Cronstein
New York University School of Medicine

Asthma as an Inflammatory Disease
Kirk Sperber
Mount Sinai School of Medicine

Theme Issues

NOVEMBER 1992

Urban Community Medicine: In Honor of Kurt Deuschle
Editors: Alan Silver and David Rose

JANUARY 1993

Issues in Medical Ethics:
1. The Physician-Patient Contract
2. Transplantation
Editors: Rosamond Rhodes and Daniel A. Moros

MAY 1993

Quality Assurance
Editor: Suzanne Kramer

OCTOBER 1993

Issues in Medical Care for Children
Editor: Richard Saphir

Available now:

Toward the Conquest of Pain
Allan P. Reed, editor
volume 58, no. 3, May 1991
84 pages **$15**

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society
Eugene W. Straus, editor
volume 59, no. 2, March 1992
96 pages **$15**

Modern Management c
Malignant Melanoma
Michail Shafir, editor
volume 59, no. 3, May 1992
86 pages **$15**

Subscriptions for 1992: $60 individuals, U.S. and Canada; $65 all libraries; $70 individuals outside U.S. and Canada.

To order subscriptions or copies of back issues ($15 each), please send check, payable to The Mount Sinai Journal of Medicine, to the Journal at Box 1094, One Gustave L. Levy, New York, NY 10029-6574; counter sales at Suite 1E, 50 E. 98th Street, New York, NY (phone: 212 241-6108; FAX: 212 722-6386).

3 4805 03620198

THE
OUNT SINAI JOURNAL
F MEDICINE

ME 59 NUMBER 6 NOVEMBER 1992

Urban Community Medicine: The Mount Sinai Experience

Honoring the Work of Kurt W. Deuschle

Alan L. Silver and David N. Rose, guest editors

From the Department of Community Medicine
Mount Sinai School of Medicine (CUNY)

Veterans
Affairs

Beth
Israel
Medical
Center

THE MOUNT SINAI JOURNAL OF MEDICINE

The Mount Sinai Journal of Medicine is published by The Mount Sinai Medical Center of New York and has the following affiliates: Beth Israel Medical Center, New York; Bronx Veterans Affairs Medical Center, New York; and Elmhurst Hospital Center, New York.

Editor-in-Chief

Sherman Kupfer, M.D.

Editor Emeritus

Lester R. Tuchman, M.D.

Associate Editors

Harriet S. Gilbert, M.D.   Julius Wolf, M.D.

Managing Editor

Claire Sotnick

Business and Production Assistant

Claudia N. Melendez

Assistant Editors

Stephen G. Baum, M.D.
David H. Bechhofer, Ph.D.
Constanin A. Bona, M.D., Ph.D.
Edward J. Bottone, Ph.D.
Jurgen Brosius, Ph.D.
Lewis Burrows, M.D.
Joseph S. Eisenman, Ph.D.
Adrienne M. Fleckman, M.D.
Richard A. Frieden, M.D.
Steven Fruchtman, M.D.
Paul L. Gilbert, M.D.
James H. Godbold, Ph.D.
Richard S. Haber, M.D.
Noam Harpaz, M.D.
Dennis P. Healy, Ph.D.
Tomas Heimann, M.D.
Barry W. Jaffin, M.D.
Andrew S. Kaplan, D.D.S.
Samuel Kenan, M.D.
Suzanne Carter Kramer, M.Sc.
Mark G. Lebwohl, M.D.
Kenneth Lieberman, M.D.
Charles Lockwood, M.D.
Steven Markowitz, M.D.
Bernard Mehl, D.P.S.
Myron Miller, M.D.
Edward Raab, M.D.
Allan Reed, M.D.
Allan E. Rubenstein, M.D.
David B. Sachar, M.D.
Henry Sacks, M.D.
Robert Safirstein, M.D.
Ira Sanders, M.D.
Martin H. Savitz, M.D.
Clyde B. Schechter, M.D.
Michael Serby, M.D.
Phyllis Shaw, Ph.D.
George Silvay, M.D.
Barry D. Stimmel, M.D.
Nelson Stone, M.D.
Max Sung, M.D.
Carl Teplitz, M.D.
Rein Tideiksaar, Ph.D.
Richard P. Wedeen, M.D.
Michael F. Wesson, M.D.

Editorial Board

Barry Freedman, M.B.A.
Richard Gorlin, M.D.
Nathan Kase, M.D.
Panayotis G. Katsoyannis, Ph.D.
Charles K. McSherry, M.D.
Jack G. Rabinowitz, M.D.
John W. Rowe, M.D.
Alan L. Schiller, M.D.
Alan L. Silver, M.D.
Alvin S. Teirstein, M.D.
Rosalyn S. Yalow, Ph.D.

The Mount Sinai Journal of Medicine (ISSN No. 0027-2507; USPS 284-860) is published 6 times a year in January, March, May, September, October, and November in one indexed volume by the Committee on Medical Education and Publications (Owner), The Mount Sinai Hospital, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Subscription price: individuals, U.S. and Canada, $60 per year; all libraries, $65; individuals outside the U.S. and Canada, $70. Single copies, $15. New subscriptions begin with the first issue of the calendar year. Send notice of change of address 60 days before the change is effective. Second-class postage paid at New York, NY and at additional mailing offices. POSTMASTER: Send address changes to The Mount Sinai Journal of Medicine, Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574. Copyright 1992 The Mount Sinai Hospital.

Critical Care America is Sensitive to Your Special Needs

Critical Care America (CCA), an innovative leader in home infusion therapy, is dedicated to providing superior quality care and support by offering specialized programs for HIV, oncology, solid organ and bone marrow transplant patients in the alternate site or home setting.

CCA offers a wide range of both traditional and innovative home infusion therapies, and complements them with 24 hour expert nursing, pharmacy, materials management and reimbursement services.

Our comprehensive programs help to meet both the needs of the patient and the needs of the physician more effectively and efficiently than any other home care provider.

Please call us toll-free for more information.
(800) 722-7003 / (800) 344-5500

Critical Care
AMERICA

When you don't know all the medicines your patients are taking, protecting their health is a crap shoot.

Counseling patients about all their medicines improves their odds of getting well and staying well.

EVERYONE WINS WHEN YOU TALK

Send me a *free* Medicine Counseling Kit.

Name

Organization

Street

City State Zip

Mail to:
National Council on Patient Information and Education
666 Eleventh Street, NW, Suite 810
Washington, DC 20001
To fax your request -- (202) 638-0773

this publication is available in microform

Please send me additional information.

University Microfilms International

300 North Zeeb Road
Dept. P.R.
Ann Arbor, MI 48106
U.S.A.

18 Bedford Row
Dept. P.R.
London, WC1R 4EJ
England

Name

Institution

Street

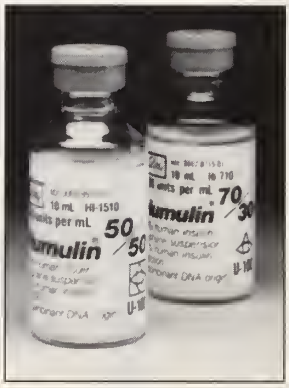
City

State Zip

Because One Size Doesn't Fit All...

New Humulin 50/50 is the tailor-made answer to individual patient needs. A unique combination of equal amounts of Regular human insulin and NPH human insulin, it will be useful in situations in which a greater initial insulin response is desirable for greater glycemic control.

Like Humulin 70/30*, new Humulin 50/50 offers the convenience and accuracy of a premix. And it can be used in conjunction with an existing 70/30 regimen.



New Humulin® 50/50

50% human insulin isophane suspension
50% human insulin injection
(recombinant DNA origin)

The Newest Option in Insulin Therapy

WARNING: Any change of insulin should be made cautiously and only under medical supervision.

* Humulin® 70/30 (70% human insulin isophane suspension, 30% human insulin injection [recombinant DNA origin]).

Lilly

Global Excellence in Diabetes Care
Eli Lilly and Company
Indianapolis, Indiana
46285

HI-7911-B-249343 © 1992, ELI LILLY AND COMPANY

THE
MOUNT SINAI JOURNAL
OF MEDICINE

Volume 59
Number 6
November 1992

CONTENTS

Urban Community Medicine: The Mount Sinai Experience Honoring the Work of Kurt W. Deuschle
Alan L. Silver and David N. Rose, guest editors

Kurt W. Deuschle and Community Medicine: Clinical Care, Statistical Compassion, Community Empowerment *Alan L. Silver and David N. Rose* 439

Kurt W. Deuschle: The Clinician in the Community *Jeffrey P. Koplan and Stephen B. Thacker* 444

The Academic Legacy of Kurt W. Deuschle *Arthur L. Frank* 447

Kurt W. Deuschle as an Actor and Community Medicine as His Stage *David E. Rogers* 450

Reminiscences of a Student of Community Medicine *Abbie Jacobs* 453

The International Program in Community Medicine at Mount Sinai Medical Center *Samuel J. Bosch, Ellen P. Fischer, Judith Freidenberg, and Rolando Merino* 455

Community Medicine: Its Contribution to the Social Science of Medicine *Samuel W. Bloom, Jane Moss, Renate Belville, Judith Freidenberg, Debbie Indyk, and Edward J. Speedling* 461

Public Health in Crisis: The Economic Consequences of Inaction *Marianne C. Fahs* 469

Community Partnerships in the 1990s: Can Urban Medical Centers Meet the Challenge? *Gary Rosenberg, Helen Rehr, and Susan Blumenfield* 479

Hepatitis B Virus Vaccine in The Gambia, West Africa: Synergy between Public Health Research and Practice *Robert W. Ryder* 487

The Epidemiology of HIV Infection and AIDS in East and Central Harlem, NY *David N. Rose* 493

continued

Volume 59
Number 6
November 1992

CONTENTS *continued*

Child Labor in the United States: Historical Background and Current Crisis *Philip J. Landrigan, Susan H. Pollack, Renate Belville, and James G. Godbold* 498

List of *Journal* Reviewers in 1992 505

Subject, Author, and Title Index to Volume 59 509

Illustrations:
Kurt W. Deuschle

On rounds, Navajo Nation, 1953 441
Portrait painted in 1968 442
In his Mount Sinai office in 1973 443
In his Mount Sinai office in 1991 443
In rural China, 1978 449
Receiving the 1989 New York City Mayor's award for science and technology 460
Enjoying goatmeat refreshments at Many Farms, Navajo Nation, 1975 486
Presenting medically equipped transport van to Harlem Triangle Association, 1970 497
In his Dutchess County, NY, garden, 1991 inside back cover

Index to Advertisers

Critical Care America page i
Eli Lilly and Company page ii

Kurt W. Deuschle and Community Medicine: Clinical Care, Statistical Compassion, Community Empowerment

Alan L. Silver, M.D.
David N. Rose, M.D.

Urban Community Medicine: The Mount Sinai Experience—Honoring the Work of Kurt W. Deuschle

The best of all leaders is the one who helps people so that, eventually, they don't need him anymore. The best leader doesn't say much, but what he says carries weight. When he is finished with his work, the people say, "It happened naturally."—Lao Tzu

Community Medicine

Community medicine studies the patterns and the social and environmental causes of diseases in population groups and seeks to develop the means by which those disease problems can be solved. It encompasses the traditional skills and knowledge of preventive medicine as well as a concern for the delivery of medical care. Grounded in clinical medicine, it incorporates elements of public health and the social sciences.

Clinical Care and Statistical Compassion. Public health is the combination of sciences, skills, and beliefs that are directed to the maintenance and improvement of the health of all the people through collective actions. Programs such as water sanitation emphasize the prevention of disease and the health needs of the population as a whole (1). The perspective of public health is purely on populations. Clinical medicine disciplines, by contrast, focus on the individual. The intent is to cure, and if cure is not possible, to maximize the health and functional capacity of individual persons. Diagnosis and management of disease are the mainstays of the doctor-patient relationship; the orientation is to each individual case.

The connection of individuals to populations and of one-to-one clinical care to health as a social phenomenon is the domain of community medicine. It is the understanding that the illness of an individual is linked to disease in a population. To learn about the distribution and determinants of disease requires looking at groups. This perspective, grouping individuals to study disease, is a central element of community medicine. Community medicine, however, starts with the individual. It is rooted in clinical care. What community medicine intends is an understanding of individuals as members of populations, and of populations as a grouping of individuals; the development of statistical compassion, for "statistics are people with the tears erased" (2).

Statistical Compassion and Community Empowerment. An epidemiologic understanding of disease can contribute much to programs of treatment and prevention. Epidemiology and clinical care, however, cannot provide health. They must be linked to community organization. Medicine is a social as well as a biological science, and medical care is a social technology. Community medicine practice must therefore also encompass anthropology, econom-

---

From the Department of Community Medicine, Mount Sinai School of Medicine (CUNY), New York. Address reprint requests to Alan L. Silver, M.D., Box 1043, Department of Community Medicine, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

ics, environmental medicine, health education, management science, sociology, and social work.

These disciplines provide tools by which individual medical care is transformed to social health. But as the focus shifts from individual biology to group ecology, science merges with collective responsibility and action. Program development must follow the interests and priorities of community residents. Communities are dynamic organisms. Community medicine works only if it is grounded in the articulated priorities of community people. The examination of the diseased individual ultimately leads to community empowerment.

Community Medicine and the Mount Sinai Concept

The concept of bridging biologic science, clinical care, and the social sciences was envisioned with the creation of the Mount Sinai School of Medicine. As articulated by Hans Popper, there would be three faculties, each contributing to the improvement of health (3, 4). The first faculty would be the basic science faculty, who would enhance our knowledge of disease and therapeutic mechanisms. The second faculty would be the clinical faculty, who would transform this knowledge into treatment for individuals. The third faculty would be the community medicine faculty, who would help identify and solve health problems of human populations.

The first president and dean of The Mount Sinai Medical Center, George James, a former New York City health commissioner and a practicing internist, sought someone who best understood this concept and could help transform it to reality. Dr. James recruited the founding father of community medicine in the United States, Kurt W Deuschle.

Kurt Deuschle and American Community Medicine

Although community medicine had been a recognized as a public health discipline in Europe, it was slow in coming to the United States. The individual generally recognized as having formulated the concept of clinical community medicine is Kurt Deuschle.

Dr. Deuschle received his B.S. degree, cum laude, at Kent State University in 1944 and his M.D. at the University of Michigan in 1948. This was followed by an internship in medicine at Colorado General Hospital in 1949 and an internal medicine residency at Syracuse from 1952 to 1954.

Applying for a position with the U.S. Public Health Service in anticipation of being assigned to India to study tropical disease, he found himself instead being assigned to the U.S. Indian Health Service and the Navajo Nation. There he was a senior assistant surgeon and chief of the Tuberculosis Program at the Navajo Medical Center, Ft. Defiance, Arizona. Dr. Deuschle later became affiliated with Cornell Medical College as an assistant professor. Working with John Adair, Walsh McDermott, and others at the Navajo Nation, Dr. Deuschle made major contributions to the field of medical anthropology, developed a prototype of community health centers, and advanced clinical care in tuberculosis, being involved in the first field trials of isoniazid.

These experiences helped formulate a vision of clinical community medicine and led to Dr. Deuschle being appointed, in 1960, to the chairmanship of the first Department of Community Medicine in the United States at the University of Kentucky. There he developed a model of how an academic medical center could promote health in a rural setting. His accomplishments at Kentucky led to his coming to Mount Sinai in 1968, as chairman of the first urban Department of Community Medicine. He stepped down as chairman in 1990 and remains in the department as an active faculty member.

Kurt Deuschle's methods of practicing the art of urban community medicine are best typified by two documents prepared by faculty members at the beginning and the end of his time as department chairman. The first is "The People of East Harlem," the report of a door-to-door survey of the health issues most important to the residents of the community, conducted in 1971 by the late Louise Johnson. Eighteen years later came the "Report on Primary Care in East Harlem," a health plan based on three years of work building a coalition among East Harlem health providers. These documents typify Dr. Deuschle's modus operandi in two ways. First, they represent his belief that a community diagnosis must be based on carefully gathered information. Second, they show that community medicine can only work if it is grounded in the articulated priorities, interests, input, and empowerment of the community.

Dr. Deuschle has been called on by government agencies, foundations, and foreign medical centers for international consultation on medical education, epidemiology of disease, and health care delivery systems in Nigeria, Vietnam, Turkey, Colombia, Jamaica, Mexico, The Dominican Republic, and the People's Republic of China.

He is a past and current member of numerous international, national, state, and local institutes, advisory panels, and committees, including the World Health Organization Expert Advisory Panel on Professional and Technical Education of Auxiliary Personnel, the Institute of Medicine of the National Academy of Sciences, the Office of Technology Assessment of the U.S. Congress, the New York City Board of Health, the New York State Council on Graduate Education, and the U.S. Preventive Services Task Force.

Kurt Deuschle's many awards include the American Public Health Association Award for Excellence in Domestic Health, the Distinguished Service Award of the American College of Preventive Medicine, the Duncan Clark Award of the Association of Teachers of Preventive Medicine, and the New York City Mayor's Award of Honor for Science and Technology.

The American Public Health Association Award citation for Kurt Deuschle said this:

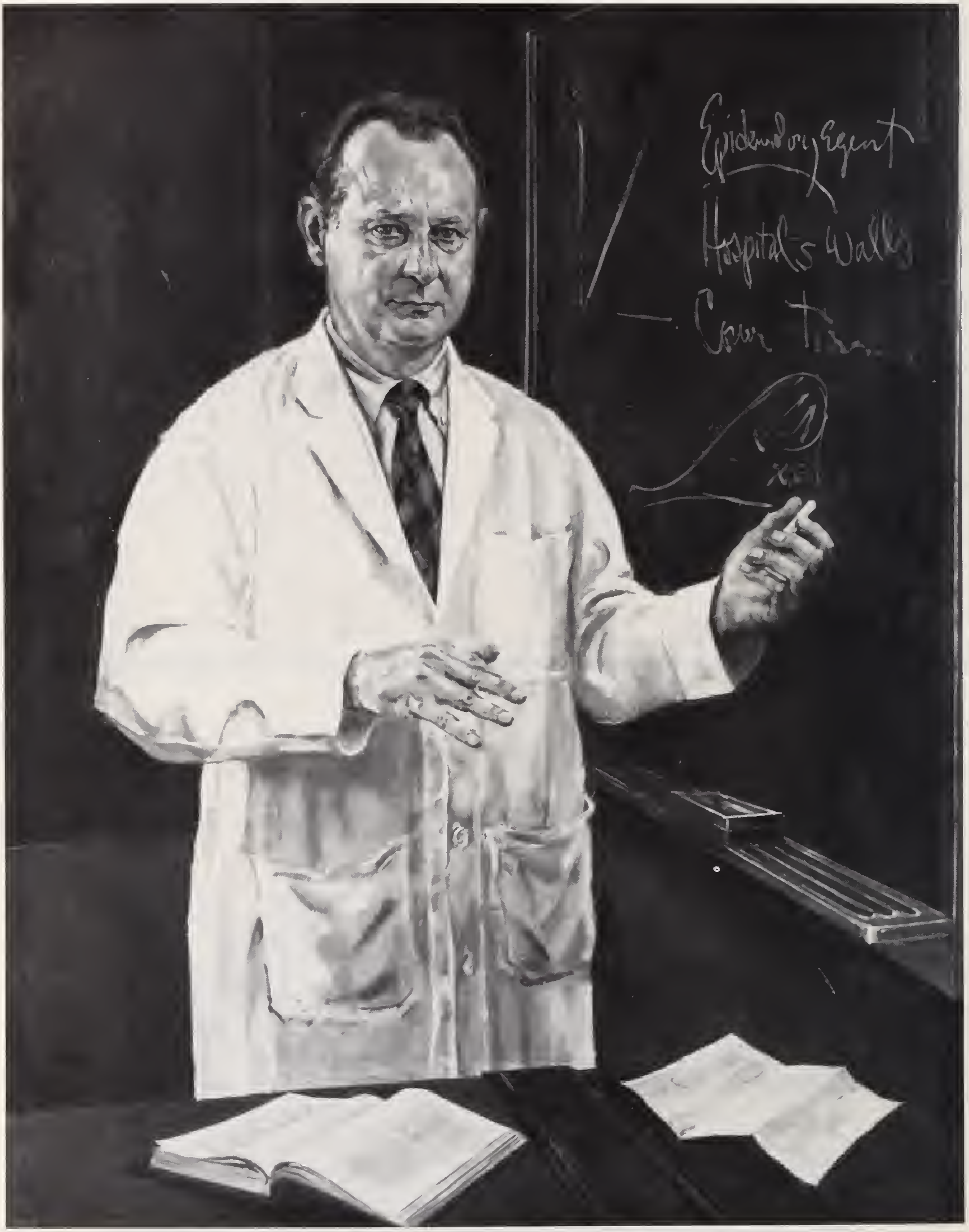
> He personally defined the discipline of community medicine in the United States. In the field at Many Farms, Arizona, he teased its basic elements from the realities of community life, and tested its principles in daily practice. At Kentucky his vision and energy firmly established its worth as an essential element in medical education. In New York City he has seen its precepts applied for good to the problems in urban settings.
>
> There are hundreds of Arizona Navajo, Appalachian highlanders, and East Harlem residents who recall vividly their personal contacts with Kurt Deuschle and are grateful for the contribution he has made to their lives. He has also been a continuing inspiration to generations of students, housestaff, and colleagues whose lives have been touched by his association and guided by his influence. Throughout the world, but especially in this country, his community medicine disciples are increasingly recognized in prominent health leadership roles which reflect additional luster upon his own brilliant career. The aggregate impact for good of Dr. Deuschle continues.

This issue gives testimony to the inspiration Kurt has been to his students and colleagues and highlights some of the current work of both the department and the vision he created. We, as coeditors of this issue honoring the work of Kurt Deuschle, are proud to work with him and to be beneficiaries of his knowledge, wisdom, and wit.

References

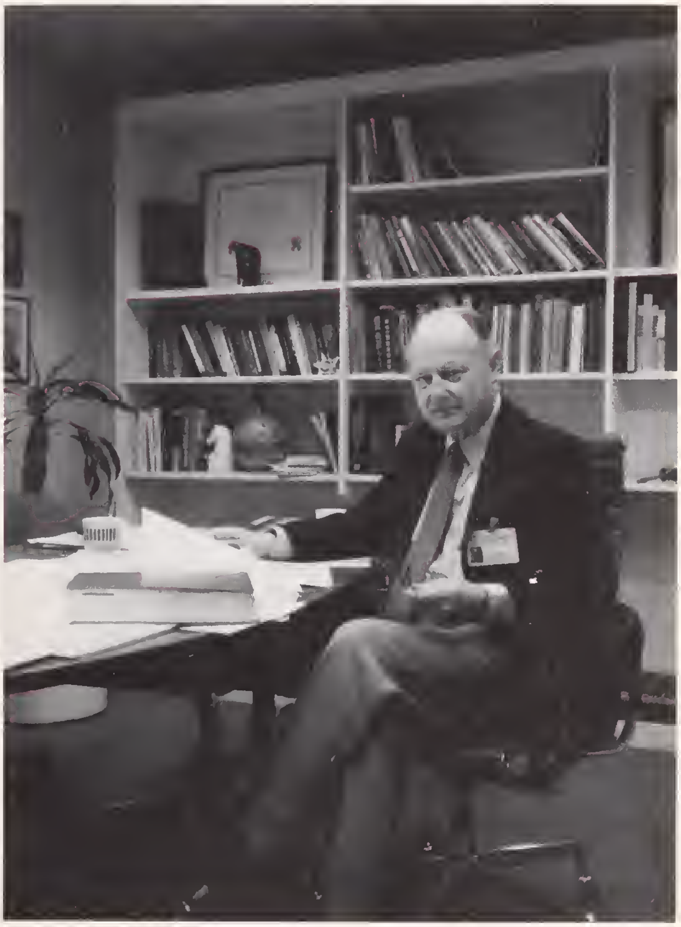
1. Last J (ed). A dictionary of epidemiology. New York: Oxford University Press, 1983, 84.
2. Mahoney M. Convocation exercise, Mount Sinai School of Medicine, New York City, Sept. 18, 1990.
3. Popper H. The Mount Sinai concept. Clin Res 1965; 13: 500–504.
4. Popper H. A new curriculum. Ann NY Acad Sci 1965; 128:552–560.

Kurt W. Deuschle goes on rounds, Navajo Nation, 1953.



Portrait of Kurt W. Deuschle painted in 1968 honoring him as the founder of community medicine. It is on display at the University of Kentucky Medical Center in Lexington, Ky.

Above: Kurt W. Deuschle in 1973 in his old office at Mount Sinai Medical Center in the Annenberg Building, working with a health facilities map of East Harlem, which is visible through the window.



Right: Kurt W. Deuschle in 1991 in his current Mount Sinai Department of Community Medicine office in the Basic Sciences Building.

Kurt W. Deuschle:

The Clinician in the Community

Jeffrey P. Koplan, M.D., M.P.H., and Stephen B. Thacker, M.D., M.Sc.

The assigned theme for this paper honoring Kurt Deuschle is "applying clinical skills to the community." This theme, however, represents only part of Kurt Deuschle's approach.

The aspects of clinical skills that fit with Kurt Deuschle's approach are those associated with the "giants" of Mount Sinai clinical medicine—those that involve deductive reasoning, empathy, judgment, intellectual rigor. He has applied such skills and attributes to community analysis and intervention.

However, Kurt Deuschle also instilled in us the need to bring the community to the clinical center and the bedside, to the academic or private attending physician, and to the health-care facility. Thus, a physician seeing a 55-year-old woman with lung cancer should look at her in the context of her social and cultural milieu, as well as with the appreciation that for every patient with lung cancer hospitalized in an academic center, there are 9 persons with emphysema, 38 with acute bronchitis, 52 with chronic bronchitis, and 320 addicted to smoking with cough or decreased vital capacity. A similar iceberg of submerged social facts exists for other conditions related to smoking and many other illnesses we encounter in the hospital—a cerebrovascular accident in a hypertensive individual, for example.

The interaction between the clinician and the public health practitioner is now more important than ever. Each participant is increasingly dependent on the other to properly fulfill their responsibilities, whether the subject is tobacco control, AIDS, or occupational health. Anticipating the need for diverse disciplines to work collaboratively, Kurt Deuschle encouraged interdisciplinary teams extending beyond physicians and nurses in public health. He had one of the first departments of community medicine that included a senior-level health economist and health sociologist. He recognized the dual need to bring the community into the clinical setting and to bring the clinician to the community. This combination is a natural blend for Mount Sinai (Fig.).

A personal note: Kurt Deuschle was a major reason that both of us came to the Mount Sinai School of Medicine—for its strong Department of Community Medicine, for its commitment to community service, and, primarily, because of him.

Community service and concern for social equality were concepts that flourished briefly, but intensely, during the late 1960s and early 1970s. Community medicine and student activities also flourished at that time, often reinforcing one another.

At one level, men and women like Kurt Deuschle, Roy Brown, and Bess Dana wanted to see that medical students gained an education in the real world—not only at the bedside, but also in the community.

For those of us who were already interested in broader issues of health in the community, Kurt offered a scientific and structured approach to the educational, research, and service programs of the community.

Kurt Deuschle: A Biographic Review

Kurt Deuschle's academic career began at Kent State University, and he followed a traditional path of medical training through Ann Arbor, Denver, and Syracuse. Like ours, Deuschle's career was reoriented by two years in the U.S.

Adapted from the authors' presentation at the symposium honoring Kurt W. Deuschle, M.D., at The Mount Sinai Medical Center on October 4, 1990. From the Centers for Disease Control, Atlanta, Georgia. Address reprint requests to Stephen B. Thacker, M.D., Director, Epidemiology Program Office, Centers for Disease Control, 1600 Clifton Rd NE, Bldg 1, Rm 5009, Atlanta, GA 30333.

Public Health Service, on assignment to the Navajo-Cornell Field Health Research Project. There he developed a lifelong relationship with Walsh McDermott and others, including former patients he has visited 20 years after their discharge from care. In 1960, Kurt made real a vision in forming the first Department of Community Medicine at the University of Kentucky. His expertise became recognized internationally, and he became a widely sought consultant whose insight and wisdom were highly valued. He then, of course, brought that vision to big-city New York in 1968. Here Kurt met and developed collaborative relationships with the leaders of the Mount Sinai community and New York City itself. Yet the man we know and admire is clearly most comfortable among the men and women who work with him daily and experience his kindness, humor, and warmth.

Kurt W. Deuschle can be best described as a mentor—a close, trusted, and experienced guide. He has been a mentor, but also a role model, a teacher, a career counselor, and an academician (as an intellectual force and as an agent of change). He stressed a need for clinical and scientific credibility as a background for public health. When we were third-year medical students, Kurt told us that surgeons make the best public health professionals because they are decisive and they are activists. He has been skilled at developing new programs with scant resources, a situation with which we are familiar at the Centers for Disease Control. He established a community-oriented program in an institution that emphasized research and clinical care. He has been a mesh of community activist/mentor/academician.

Deuschle's Dicta

We left Mount Sinai School of Medicine not only with assorted facts and technical skills, but also with a set of principles and a role model for our subsequent careers. From Kurt we have distilled in our own minds four guiding principles. We have termed them Deuschle's Dicta.

Dictum 1: Be Grounded in a Clinical Specialty. Kurt strongly counseled his students, especially those interested in social and policy issues, to focus their energies on mastering clinical skills before setting out on their life's mission. Even when students were impatient with delaying their desires to rush to more political- or policy-oriented activities, Kurt's gentle arts of persuasion and wisdom prevailed. His effectiveness as a mentor was demonstrated in class after class of young students. For those of us deeply interested in community issues, he emphasized the need for us to excel as clinicians to assure credibility with our peers in the professions. He also counseled us to remember our own humanity and recognize our limitations. We, as physicians, will make mistakes. Sometimes a mistake will have a tragic effect on a patient. For those of us who saw the patient as the community, such mistakes would not be buried; rather, they would be magnified in impact.

Community and Hospital at Mount Sinai

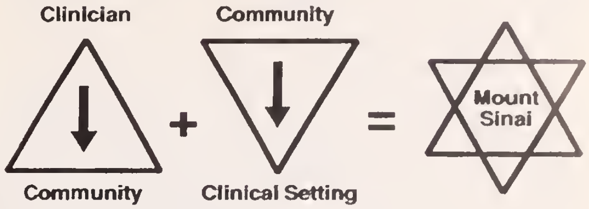


Fig. The Deuschle model.

As a result, one of us (S.B.T.) went to Duke to study family medicine and to train as a Robert Wood Johnson Clinical Scholar, and the other (J.P.K.) went to Montefiore and Stanford to study internal medicine. Kurt pointed us toward the Centers for Disease Control after Mount Sinai for community experience with a quantitative base. Thus, after Duke, S.B.T. became an Epidemic Intelligence Service officer assigned to the Washington, D.C., Health Department; J.P.K. entered the same service to serve in the Smallpox Eradication Program with duty in Bangladesh. Cross-cultural experiences have taken us to extended assignments and projects in Trinidad, England, Egypt, and China, as well as short-term consultancies throughout the world. These were our career paths that parallel Kurt Deuschle's Navajo, Kentucky, and Mount Sinai experiences.

Dictum 2: The Community Is Your Patient. Go to a community, study it. Observe, analyze, publish, and act. Publication and action are rarely paired in a world where these activities tend to be mutually exclusive. Kurt made us think our role was incomplete without the intellectual rigor of publication and peer review. Requiring publication argues that if something is worth doing, it is worth doing well, and also that you are more likely to do it well, and do it analytically, if you anticipate writing it up. At the same time, the public health professional must be more than chronicler, documenter, and diagnostician; he or she must intervene to treat the community.

This concept, however, has dogged both of us, leaving investigations and accumulated datasets hanging over our heads with the sense of a job unfinished. Eventually, we have finished those pieces driven by a professional superego instilled and bestowed by Kurt.

Although we professionally classify ourselves as epidemiologists-cum-public health specialists (subclass federal bureaucrats), we did seek an environment that permitted, and, indeed, encouraged, the Deuschle mix of intellectual rigor and activism. We found this at the Centers for Disease Control and have 32 years of experience there between us. It is also fitting that Kurt's successor—that great Celto-Semite Philip Landrigan—should represent this same tradition.

Dictum 3: Leadership Is an Opportunity and a Responsibility. If you think you are right, think big and take a national leadership role. Kurt Deuschle did it in community medicine, medical education, and community-oriented primary care. We are trying to do our part in public health, in particular in epidemiology, chronic disease prevention, and health promotion.

The Epidemic Intelligence Service each year provides more than 60 young men and women with the epidemiologic tools of the community clinician, taking a health problem ready for an intervention, particularly one affecting the poor and disenfranchised, and attacking the problem.

For the past several years we at the centers have been working to further develop a stronger national public health approach to chronic diseases, such as breast and cervical cancer, diabetes, heart disease, and tobacco use, and their risk factors.

The responsibilities of leadership take many forms. For us, beyond our regular work in public health, we perceived a need for leadership in another area—technology assessment. We both developed this interest while at Mount Sinai and have pursued it. J.P.K. contributed to the landmark text for the Institute of Medicine, *Assessing Medical Technologies,* and has applied decision analysis to vaccine-preventable diseases and other areas of public health. Since coming to the centers, S.B.T. has collaborated with a Mount Sinai School of Medicine faculty advisor, David Banta, on the assessment of obstetric technologies (an area in which we were as warmly welcomed initially as Deuschle's ideas of community medicine were welcomed to academic clinical medicine). Subsequently, S.B.T. has learned the tools of meta-analysis, a field pioneered by another colleague of Kurt's, a Mount Sinai giant, Tom Chalmers. Now, we are both working with Bill Roper, the new director of the Centers for Disease Control, to bring these efforts in medical effectiveness to the prevention arena.

Dictum 4: Listen to the Community and Be Responsive. Know who your constituents are, listen to them, and do not lose sight of your service responsibility. Kurt Deuschle and Walsh McDermott at Many Farms characterized the "hidden component" of health care, the cultural, social, and environmental factors that influence a community's health status. Kurt wrote in a paper in *Science* in 1972 that this sociocultural "substrate determines the limits of biomedical technology in a community; it also determines how the physician allocates his time." One of our major activities in health promotion at the Centers for Disease Control is to assist communities in analyzing their problems and choosing priorities. Our Planned Approach to Community Health (or PATCH) program lets community members engage in this process and is the foundation of most of our community programs.

The Centers for Disease Control has begun to work in the area of disability prevention and to collaborate on health promotion and disease and injury prevention with members of what is, in fact, another community within our nation—persons with disabilities. This work presents an important challenge to the clinical and public health community. In 1990, the President signed into law the Americans with Disabilities Act. We will be wise to heed Deuschle's dictum 4 as we address the health implications of the full integration of this community into our society.

There is a danger that you, our reader, will conclude from this summary of a mentor's influence on his professional offspring that we now share his stature, as well as his values. We formally and sincerely disclaim any such notion.

Kurt's grace and wisdom, his insight, kindness, and humor, his loyalty to and his intense concern for and understanding of his students and colleagues are, if not unique, at least rare features for a professor, or even a civil servant. His influence extends far beyond those of us paying tribute to him here. Kurt Deuschle has influenced many more students than the relatively small number who entered careers in community medicine and public health. He has enabled his students to see their patients in the context of a community, to be clinicians to the community.

For this, Kurt, we thank you!

The Academic Legacy of Kurt W. Deuschle

ARTHUR L. FRANK, M.D., PH.D.

FOR ME this privilege of sharing some thoughts on this festive and academically special occasion is very much a homecoming—although parts of me have never left. Not a day goes by that I do not draw on one or another experience from my time at Mount Sinai—a period that spanned 1968 to 1983, as a medical student, graduate student, house officer, and faculty member. Many of my colleagues had an influence on me, but I must single out Kurt W. Deuschle.

He has had a significant influence on many in this country. One of the first communities he worked in was the Navajo Reservation, while in the public health service in the early 1950s; he began leaving a written record for others to learn from then. From Cornell, where he served on the faculty in the late 1950s, he was selected at an early stage of his career to begin a brand-new department in a brand-new medical school, and in that setting he left a legacy that continues to be influential.

It was Kurt Deuschle who first used the term now used by more departments than any other: community medicine. In 1960, the then-new Medical School at the University of Kentucky in Lexington, which has as a primary mission serving the underserved of Appalachian eastern Kentucky, opened with Kurt Deuschle as chairman of the Department of Community Medicine.

That department quickly developed a national and international reputation. Students, house officers, and faculty from that department now hold leadership positions in the field throughout this country. That legacy, and the love and admiration for Kurt Deuschle from that time, is still palpable today. He worked at the national level, too, influencing organizations such as the Association of Teachers of Preventive Medicine, which in 1990 accorded him its highest honor.

While in Kentucky, Kurt Deuschle worked most in the Appalachian community in the eastern part of the state. Today issues of access and the quality of health care are still important problems, and significant rural poverty continues.

Having seen urban poverty during my time in New York, I find rural poverty lacks the hard edge and constant violence of the urban setting. The 100-year-old cycle of boom and bust in the coal fields has turned now into permanent bust, since even if coal returns more strongly, people have been replaced by machines and fewer workers are now mining more and more coal. This permanent poverty, coupled with a lackluster education system, are the reasons why the problems of Appalachia have been so recalcitrant.

In Kentucky one is also struck by the high degree of truly preventable disease, be it tuberculosis, cervical cancer, or water-borne infections, or that problematic nondisease, early teenage pregnancy. Overlying these more traditional concerns are complex new ones, including groundwater contamination, the importation of hazardous materials (including nuclear waste), and the occasional overwhelming power of nature in the mountains, be it flooding of flatland, the washing out of a bridge, or rockslides.

Culturally, working there requires adjustments. After having had to learn some Spanish to practice at Mount Sinai, I am now learning what is occasionally almost a foreign language, talking "southern," which I need to do to communicate

---

Adapted from the author's presentation at the symposium honoring Kurt W. Deuschle, M.D., at the Mount Sinai Medical Center on October 4, 1990. From the Department of Preventive Medicine and Environmental Health, University of Kentucky. Address reprint requests to the author, Professor of Preventive Medicine and Environmental Health, 800 Rose St., Lexington, KY 40536-0084.

well with many of my current patients. The sense of family and geographic roots are much felt in the mountains.

Having had a significant influence in Kentucky, Kurt Deuschle then undertook the equivalent urban challenge of bringing change to underserved urban communities. He became the first chairman of the Department of Community Medicine at Mount Sinai and early on was named to an endowed chair and served with distinction as chairman until 1990.

My own first meeting with Kurt Deuschle was in 1968, in the days just after the Mount Sinai medical school opened, when I had the joy of being in the first first-year class, the class of 1972. During my 15 years at Mount Sinai, several individuals strongly influenced me, but I learned several important lessons best from Kurt Deuschle, often by example.

I learned what it really meant to be a teacher when Kurt shared with me that his goal was to teach a student everything he knew and hope that the student went him one better. I had a role model for how to be responsible for many colleagues in a department and how, as a professional equal, to stand on principle in dealing with deans and presidents for what one felt was right. Importantly, and almost uniquely, Kurt demonstrated how one could lead without bombast and without the attitude of superiority that one often sees in academic medicine.

The most important lesson in our discipline was to think in terms of populations but, at the same time, never to lose sight of the care that physicians show by their work. This caring approach can easily be taken in the usual settings for practice. After all, Kurt first trained as an internist and knew about one-on-one medicine, but now he had us learning about "statistical compassion"—seeing beyond the aggregate numbers and remembering the suffering of fellow humans who make up the statistics.

I left Mount Sinai in 1983 to become chairman of Kurt's old department at the University of Kentucky, now renamed the Department of Preventive Medicine and Environmental Health. The intervening years had not been kind to the department. After its spectacular early success under Kurt, it had fallen on hard times. By 1983 it had actually been closed for several years. It had been renamed, and plans were formulated to focus on environmental issues as well as its more traditional work; thus there was a real challenge waiting for me in starting from scratch. In fact I did not start quite from scratch; although Kurt Deuschle had been gone for 15 years when I arrived, being one of "Kurt's boys" was a great leg up—and in horse country, that kind of assistance is indeed important.

There is still much love and affection for Kurt Deuschle in Kentucky, from biochemistry professors and anthropologists, to the director of the Aging Center, who was in the first graduating class of the University of Kentucky, to the current dean, who is another University of Kentucky graduate from Deuschle's time.

One now wanted to follow in his tradition, as one set about reconstituting work in communities, and the term "communities" was to be broadly defined. The medical school is now reexamining its roots, having just begun a major rural health initiative to impact on eastern Kentucky, where there continue to be many unmet health needs. My own current department will have a major role in these developments. We also expect a major role in the curriculum reassessment, funded by the Robert Wood Johnson Foundation, going on at the school.

Like Kurt Deuschle, I, too, had trained as an internist, in the great tradition of the Department of Medicine of The Mount Sinai Hospital. I continue to spend much of my time involved with clinical work, especially in my own chosen area, occupational and environmental health. In the Deuschle tradition, however, the communities in which one can work can be quite varied—from a statewide program to a single workplace. A few examples may suffice to illustrate this diversity:

- A recent grant award by the Agency for Toxic Substances and Disease Registry has mounted a joint effort between our department and the State Health Department to educate all 6,200 physicians in Kentucky on occupational and environmental health matters and to set up an 800-number telephone service for consultation and professional advice.
- A National Institute for Occupational Safety and Health farm injury surveillance cooperative agreement award is furthering statewide study of agricultural-related illness and injury in direct cooperation with the agricultural extension service in the College of Agriculture. Kurt especially will recognize this as the farm extension model.
- Leadership exists in our department for acquired immunodeficiency syndrome education to health professionals throughout the state.
- Service to corporations, such as Toyota, IBM, Ashland Oil, and Cyprus Minerals, includes providing workplace-related health services. At Cyprus Minerals, this service, now in its

sixth year, involves the care of 800 coal miners in Hazard, Kentucky—an appropriate name for such work—where the traditional workplace physical examinations for employment, black lung, and injuries have been expanded into wellness activities—weight reduction and smoking cessation programs and on-the-job exercise facilities.

- Work with labor unions concerned about asbestos hazards in the construction trades or musculoskeletal problems in manufacturing plants.
- The department has not forgotten its own "community," the University of Kentucky. We are the largest employer in Lexington and we render service within the university. Lexington is a community of 220,000, much changed from Deuschle's time there. One can now get *The New York Times* on the day it is printed, and fresh bagels at the Manhattan Bagel Company.

Following in the Deuschle tradition of education, we train medical students and residents, and three years ago began a Master of Science in Public Health degree program, in which student enrollment is now climbing. Next July we plan to formally initiate training in industrial hygiene, although we already have students pursuing that track. The working community of Kentucky is in great need of such individuals. Future expansion may take us into occupational health nursing and graduate programs for occupational physician assistants. Our own departmental industrial hygienist serves as a technical advisor to the university, including the main campus and contiguous medical center, as well as the 14 statewide community colleges.

It reflects on Kurt Deuschle and his legacy at Mount Sinai that the Centers for Disease Control is well represented in this symposium by Jeffrey Koplan and Steve Thacker, both contemporaries of mine. Kurt Deuschle has influenced us all. The most prestigious alumni award of The Mount Sinai School of Medicine is the Horowitz Award, for which only those who graduated at least five years before are eligible. Of the 15 or so recipients since that award was initiated in the mid-1970s, three—Jeffrey Koplan, Steve Thacker, and myself—are represented in this symposium honoring Kurt Deuschle, and a fourth participant, Abbie Jacobs, has not yet received it only because, I am convinced, she has not yet been out of school for five years. That is one more sign of Kurt Deuschle's academic legacy.

Kurt W. Deuschle in rural China, 1978, reviewing the development of barefoot doctors as a consultant for the U.S. State Department.

Kurt W. Deuschle as an Actor and Community Medicine as His Stage

David E. Rogers, M.D.

A statement from my heart: What a privilege, what a treat, to participate in honoring my wonderful long-term friend and colleague Kurt Deuschle. This is indeed a celebration, and well it should be. We are paying tribute to a modest, warm, understated man who has been a joy to all who know him. He is an imaginative, humanistic visionary and he has been a leader in the movement to give community medicine its rightful place in the sun. He has had a remarkable career, and I feel fortunate to have grown up in medicine with him.

My one qualification for participating here—for I am a humble, conservative, card-carrying internist in this thicket of wild-eyed, community-oriented healthnics—is that I have known Kurt Deuschle for 35 years. We both entered the New York Hospital scene together in 1955—he coming to work with Walsh McDermott from upstate New York, I returning to take McDermott's former position as head of infectious disease from a fellowship with René Dubos at the Rockefeller Institute. We were both powerfully shaped by our contacts with and love for Walsh McDermott. We bonded swiftly. We shared experiences in the laboratory. We got well acquainted on the Navajo Reservation, where Kurt Deuschle did such a remarkable job. We both initiated departments in emerging schools in the south—I at Vanderbilt University and he at the University of Kentucky. We both left the south at the same time—he to return to Mount Sinai in New York, I to Johns Hopkins as its dean. We have always worked on surprisingly similar agendas, and both of us have always shared a similar value system. It is a simple-minded one, for we are both simple-minded men. It is a belief that this nation can develop an effective, responsive, and humane system of basic health care that embraces all its citizens, a system that can include not only those of us who are quite fortunate, but also those who are poor, who are people of color, and who are have-nots.

Kurt Deuschle has been a consistent and articulate champion of the needs of the medically underserved. He first demonstrated that with the development of a most unusual health care program for the Indian population on the Navajo Reservation, where he was beloved. He refined it in Kentucky, where he developed a remarkable rural health program heavily involving students and physicians throughout the territory in ways that made his program vital and vibrant. He has tried the same in East Harlem, where he has been consistently at the barricades for the last 33 years. Would that there were more Kurt Deuschles in our society. If we could clone him more widely, we would have both better health care for the least served and a friendlier, happier society.

So, Kurt Deuschle, I salute you. I remember you when you were an awkward, modest young man from coal-mining country rather than a senior statesman in medicine. I have learned much from you, and I like being part of this occasion.

Why Hasn't Community Medicine Come of Age?

The original celebratory symposium held in 1990 was entitled "Community Medicine Comes of Age." And here, to my sorrow, I must strike my only discordant note. I disagree that it has "come

---

Adapted from the author's presentation at the symposium honoring Kurt W. Deuschle, M.D., at the Mount Sinai Medical Center on October 4, 1990. From the Department of Medicine, Cornell Medical College, New York, NY. Address reprint requests to the author, The Walsh McDermott University Professor of Medicine, Cornell Medical College, 1300 York Avenue, No. A-127, New York, NY 10021.

of age," and I bet Kurt Deuschle does too. The kind of coherent, well-linked system of basic community health services he has so long espoused has not come into widespread being in this country, and indeed in many ways we seem to be farther from it than we were a decade ago. Eight years ago, when I was deeply involved in trying to encourage national movement in this direction, I said some things that I cannot resist repeating now:

> Community oriented care programs are a rational way of delivering the fruits of biomedical and behavioral scientific knowledge to those who need them. No thoughtful person can argue with the logic of this approach. How can one believe that improvements in health can occur without thorough epidemiologic knowledge of health problems that exist in a community or a society? How can preventive, rehabilitative or curative services be fully effective if one knows little or nothing about the cultural, educational, economic, or psychological factors that can create barriers to care, isolate groups, and increase personal health risks for community inhabitants? No one in modern society can believe that a physician working in isolation and simply treating those who come to him on a one-on-one basis can produce a healthy society.

I went on to indicate that if that was the case, why should the following be true? And here I deliberately overstated my questions to emphasize my points:

First, why hasn't community-oriented care swept like a tidal wave over the world as an idea whose time has come?

Second, why are most of our major medical schools continuing to turn out large numbers of specialists, despite enormous evidence that what is needed are more generalists to provide community care services?

Third, why are departments of community medicine usually relatively weak forces in their academic institution, and why do they seem to have so little impact on medical education in general?

Fourth, why have the mountains of literature on community-oriented primary care written during the last decade had so little impact on how medicine is organized, taught, or practiced in this country?

Barriers to Community-Oriented Care. But I did go on to indicate what I felt were the barriers to the acceptance of your vision, Kurt, suggesting that there must be some compelling, non-health-related reasons for the failure of community-oriented care to capture the hearts and minds of health professionals. The barriers as I saw it then were the following:

First, the reward system, which did not encourage community-oriented approaches—a reward system which sent strong signals to physicians to subspecialize and maintain high-technology practices.

Second, I suggested, perhaps as part of the first, a prestige factor. I indicated that high-technology physicians and the high-technology institutions in which they worked were like space centers and the high-technology physicians like astronauts. I said that community physicians were viewed like those who drove the buses and subway trains. Although they were of enormous importance in making the everyday world work, they got few medals for the task or for making it run efficiently, affordably, or on time. Departments of community medicine to me seemed to be caught in the bind of not seeming to be where the excitement was.

Finally, I suggested that community medicine had a problem with image because of the lack of appeal of the aggregate vital statistics they used as their directional signals. I felt that the erasure of the human element—the translation of infant mortality or life expectancy into numbers on a page—loosened the grasp on public attention. I pointed out that while a community was willing to instantly galvanize to send millions of dollars to rescue a trapped coal miner or the survivors of a downed plane, that same community would only much more reluctantly spend similar sums to reduce aggregate infant mortality.

All those problems are still much with us. Indeed, I feel there has been significant slippage over the last decade. We are just emerging from an embarrassing period in this country in which I feel selfishness, greed, and blaming the victims for their ignorance or poverty or isolation from the rest of us became an accepted national excuse for not caring for our less than fortunate.

So what might we do to create the kind of community-oriented care system that Kurt Deuschle has championed—systems that can move all sick people smoothly from simple, basic care services to high-intensity in-hospital services, to nursing homes, to residential units, to hospices, and the like?

How the Barriers Might Be Removed. A few suggestions on how we might move in these directions, which I think I have learned in part from the man we honor here:

First, we clearly must restructure the reward system for health professionals. If community-oriented systems of care are to flourish, there will have to be changes in the way physicians are reimbursed for their services. Making financial incentives neutral with regard to the use of technology would, I believe, encourage more physicians to consider how their time and effort might have the greatest effectiveness in reducing human suffering. I would like to allow physicians to practice in the kind of style that seems most appealing to them without financial penalties. The new resource-based reimbursement schemes proposed by Bill Hsiao look promising.

Second, we should follow the example of our Canadian neighbors and link academic health centers closely to their communities, and graduate medical training to medical school training, so that the community responsibilities and the social contract of physicians cannot be ignored by our training centers. Separating the responsibilities for communities and patient care from the training of young men and women to be doctors, nurses, or other health professionals has permitted those of us in academe to become detached intellectually and emotionally from those we care for. Academic centers are not fulfilling their contract with the society that has supported them so well. We simply must correct this. We must be more responsive to community needs.

Last, the leaders of community medicine must develop an easy-to-grasp, more compelling database to make the case for your vision of good community care. One of the problems I alluded to earlier is that the kind of statistics currently used to track the progress of the personal health care system do not monitor with any sensitivity the changes that are reflected by most of what doctors do for sick people. Death rates, morbidity data, and other sets of current statistics simply do not measure what health professionals aim for, or the potential capabilities of community medicine.

A simple example to expand and explain this point: Thanks to modern medicine, many people with serious arthritis can now be helped to maintain relatively pain-free, independent, productive lives. But nowhere on the scorecard of health statistics does this important gift to suffering people or the health professional's role in making this possible become apparent. I would like to see a scorecard developed that would show how we in health care do for people who have diseases that do not kill but do cripple.

To return to my example: 18 million Americans are afflicted with arthritis. Almost 14 million indicate that they are bothered some or a great deal by their disease. Three million, or 18%, cannot function at all because of pain or limitation of activity.

Clearly, we should continue to work to unravel the causes of arthritis and prevent it. But I would like to see a scorecard that would show how effective we have been in reducing the problems of that 18% to some more acceptable number by good medical care. Have we dropped it to 10%? To 4%? What have health professionals done to bring these people back to an effective, pain-tolerable, functioning life?

Medicine simply must get a clearer idea of, and give a clearer presentation about, what ingredients, human and technical, assembled in what ways, can most effectively help people to maintain maximum human function, prevent functional breakdown, or swiftly restore it. We need to take that data and put it down in ways that have real emotional meaning for physicians, for health planners, and for those who are responsible for how health dollars are deployed.

In my judgment there has never been such a crying need for locally based, community-oriented systems of care as we have today. The transmedical problems that plague us—drug use, teenage pregnancies, violence, acquired immunodeficiency syndrome—are threatening to destroy us. If we are to deal more effectively with the health and medical problems of the poor or the culturally isolated or the alienated, locally designed, community-based medical care programs seem the way to go to make this a kinder and gentler and better society.

Kurt Deuschle has been preaching this for years. The way to truly honor him would be to roll up our sleeves, get out there, and make it so. So, again, Kurt Deuschle, I thank you for letting me share in this celebration of your contributions.

Reminiscences of a Student of Community Medicine

Abbie Jacobs, M.D.

Many people have known Kurt Deuschle longer than I have, but it only takes a moment to feel the presence of this exceptional man. I am privileged to honor him here. Kurt Deuschle built a strong Department of Community Medicine, taught me a great deal, and created the most significant experiences of my medical school training. He profoundly influenced my years at Mount Sinai and my choice to be a family practitioner. I still remember the discussions that I had with him, how thoughtful he was, and how he made me feel important. He has been an inspiration to me always.

My introduction to the Department of Community Medicine came during my first semester at Mount Sinai. Sam Bosch, the deputy chairman, was teaching an elective on health care organizations. I was the sole participant. Week after week, I would look forward to walking down the halls of Annenberg 10 into his office. When I entered the office, I knew that I would learn in a way that was different from the rest of my medical school classes. Bosch would draw simple diagrams on the board, relaying complex and detailed information. I would ask questions, actively participating in my education. To a first-year student feeling the pressure of acquiring vast amounts of medical knowledge and feeling anonymous as just another student, this weekly routine was a welcomed respite from my daily routine. The opportunity to learn directly from a professor kept the joy of learning alive for me.

Toward the end of my elective, I spoke to Bosch about my desire to experience medicine in a rural community. He suggested that I meet with Kurt Deuschle, the chairman of the department. It took only one meeting for me to know that Kurt Deuschle is a special person. Whether you are a first-year medical student, an established physician, or not in health care at all, you feel important when you spend time with him. We discussed the possibility of working in rural Kentucky or with the Indian Health Service, two places where Kurt Deuschle has certainly left his mark.

Since a fourth-year Mount Sinai student had just returned from the Papago Indian Reservation and the medical director was interested in having other senior students work there, we pursued that course. Although Kurt Deuschle knew I was just finishing my first year, he had confidence in me and helped me arrange to work on the Papago Reservation. I would assist in the implementation of a comprehensive diabetes program. Diabetes mellitus is a major health concern for the predominantly obese Papago Indians. This tribe lived as hunter-gatherers in earlier times, but now they lead a sedentary life. The convenience stores, poor living conditions, and dependence on the Bureau of Indian Affairs have done little to benefit the health of this tribe.

For three days, as I sat anxiously on a Greyhound bus bound for Tucson, Arizona, I never imagined that my time with the Papagos would be the most memorable experience of my medical school training and would shape my decision to be a family practitioner.

When I arrived in Tucson, I was met by an Indian guide who drove me 60 miles southwest to the capital, Sells. The beauty of the Sonora Desert with its giant saguaro cactus, prickly pear, and jumping cholla trees filled my sight. It did not matter that summer is not the ideal time to visit the American Southwest because it is monsoon

---

Adapted from the author's presentation at the symposium honoring Kurt W. Deuschle, M.D., at the Mount Sinai Medical Center on October 4, 1990. From the Bronx Lebanon Family Practice Program. Address reprint requests to the author, Curriculum Coordinator, Dept. of Family Practice, St. Mary Hospital, 308 Willow Ave., Hoboken, NJ 07030.

season. The people I met were so friendly and the environment so different that the inclement weather only added to the experience.

Working with the Papagos left a tremendous imprint on me. I saw physicians who were attuned to the cultural and sociopolitical issues affecting the people they served. Not all the doctors were trained as family physicians, yet they had to attend to all areas of medicine. The physicians took care of families, including pregnant women, children, and the elders. A physician could not ignore any factors that have an impact on a person's health. This is what I continue to see today in family practice.

While I was on the Papago Reservation, I had the opportunity to work with tribal nutrition counselors. The counselors taught their fellow Indians about diabetes. We would travel throughout the reservation, which was approximately the size of Connecticut. In some of the remote villages, there were outhouses and minimal electricity. The nutrition counselors brought posters and used a 16-mm film to teach the Papago about diabetes. Unfortunately, all the materials were Western and not culturally appropriate. The workers often communicated in Ood'ham, the native language, but the concepts were clearly "Anglo," the Indian expression for whites. For example, the film, made in the 1960s, featured white men talking in English about diabetes, which was not effective. Culturally appropriate patient education materials were needed.

The following summer I returned to Sells with a fellowship to study Papago beliefs about diabetes as a first step to developing better teaching tools. My first questionnaire was an ambitious undertaking. I wanted to know what the Papago thought about why they get diabetes, where it comes from, and what they think causes it. In addition, I included questions about access to care, diet, and exercise. The tribal nutrition workers translated the questionnaire from English to Ood'ham and frequently administered it verbally. We pretested the questionnaire first and then modified it. When I returned to New York, I analyzed the data for my third-year Community Medicine clerkship project with the help of two faculty members in the department, Judith Herbstein and Edward Speedling. Not surprisingly, I found that the dietary habits, life-style changes, and lack of understanding of diabetes as a disease greatly affected the Papago Indians' abilities to control their health. For me, working with the tribal nutrition workers and then returning to New York and working with wonderful faculty made my first "research" experience tremendously exciting.

When deciding on my career choice, I was certain that family practice would be perfect for me. I could work with people of all ages and have the opportunity to learn how different systems, the family, and the community, for instance, affect each individual. My work with the Department of Community Medicine and with the Papago Indians laid the foundation for my decision.

I did my residency training at Bronx Lebanon Hospital, which had a newly formed family practice program. The idea of a new program where I would have a great deal of input appealed to me. We were creating something. I also wanted to work in a community that was underserved and in need of good medical care. I have found working as a family physician in the South Bronx to be rewarding and challenging.

The South Bronx conjures up images of burned-out buildings and racial tension. There is repeated violence, drug use, teen pregnancies, and AIDS-related illnesses. But beyond this despair, there are wonderful families trying to survive and live in oppressive situations. You cannot work in this setting without having a community perspective and understanding the community ills.

In our Family Practice Center, I see a wide range of patients from the young to the old. Often the children are those I have delivered, and I enjoy watching them grow. I also do administrative work as the Associate Residency Director. As I teach residents and medical students, I hope that I am a role model for them as Kurt Deuschle was so importantly a role model for me.

Kurt was instrumental in creating the opportunities that enabled me to choose my career as a family physician. Although family practice may not be highly visible in New York City, Kurt Deuschle has always acknowledged the role of family practitioners in delivering health care. He is a rare blend: a man with a global vision and a true understanding of medicine, both as an institution and as a caring profession. I am grateful that he sparked my learning in medical school and paved a path for my career. I feel extremely privileged to be invited to honor this wonderful man who I so admire, and to be able to publicly tell him, "Thank you for all you've done."

The International Program in Community Medicine at Mount Sinai

SAMUEL J. BOSCH, M.D., ELLEN P. FISCHER, PH.D., JUDITH FREIDENBERG, PH.D., AND
ROLANDO MERINO, M.D., M.P.H.

THE IMPERATIVE of contributing to the development of local and regional health planning has dominated the thinking of the Department of Community Medicine since its inception. As a result, Kurt W. Deuschle, first chairman of the department, discouraged the involvement of faculty in projects abroad during the first ten years. This policy was based on the principle that first priority should be given by the school to serving its immediate surroundings, the East Harlem neighborhood in New York City (1).

By 1979, when East Harlem programs were firmly in place, Kurt Deuschle knew that the department was ready for international responsibilities. In response to a request that came through Mount Sinai's president, the department assisted a corporation in the Dominican Republic to reorganize a comprehensive health system for 80,000 people in the eastern region of the country (2). The successful outcome of that project led to the endowment of a chair in international community medicine within the department (the Charles G. Bludhorn Professorship in International Community Medicine, established in 1983).

The first professor to hold this chair was invested in 1983 and was given the responsibility for organizing an International Division of Community Medicine as its operational unit. The purpose of this paper is to present the goals of the international program and summarize the major activities meeting those goals from 1983 to 1991.

Goals and Operational Policy

The overall goal of the program is to promote community development outside the United States through improvement in health and health care delivery systems. A multidisciplinary faculty team worked closely with the division chair. The team defined program goals and an operational policy congruent with those of the department. Formal agreements that the medical school had reached with the donors of the chair were carefully integrated.

It was decided that the division would foster systematic international exchange of community medicine expertise among institutions in the United States and abroad and advance the establishment of constructive, cooperative linkages among countries. More specifically, it was agreed that the division would collaborate with organizations outside the United States with three goals in mind: (a) to enhance health planning and education activities that promote equitable distribution of resources; (b) to foster professional development in the areas of population-based health planning, primary care organization, health promotion, and health services administration; and (c) to develop a community-oriented health services research capability.

In its collaborative agreements with agencies outside the United States, the division would be guided by the following five principles:

- Division staff should play a professional advisory role and not accept responsibility for the actual administration or operation of a project.
- The prerequisite for successful cooperation in any project should be the involvement of local leadership. Collaboration with committed individuals with actual or potential leadership ca-

---

From the Department of Community Medicine, Mt Sinai School of Medicine. Address reprint requests to Samuel J. Bosch, MD, Prof. Emeritus, Community Medicine and International Health, Dept. of Community Medicine, Mt Sinai School of Medicine (CUNY), Box 1043, One Gustave L. Levy Pl., NY, NY 10029.

pabilities within an organization was considered key to the development of self-sustaining programs.

- Priority should be given to cooperative arrangements with universities, particularly those with schools of health sciences.
- Priority should be given to collaboration with institutions that take a population-based approach to problem identification and resolution.
- Priority should be given to programs where the responsibility to serve a defined population group is assumed by an interdisciplinary team.

Because the Mount Sinai School of Medicine is located in a predominantly Hispanic community (East Harlem), and the department already had many faculty who were bilingual and conversant with Hispanic cultures, it was decided that geographic priority would be given to requests emanating from Hispanic countries in the Americas.

Most medical schools in the Americas belong to national associations (such as the Association of American Medical Colleges) that cooperate internationally through the PanAmerican Federation of Associations of Medical Schools. For activities in the Americas, preference would be given to programs carried out in coordination with this federation (A. Mejia, personal communication, October 1983).

Programs and Activities

Since 1983, the division has coordinated collaborative efforts between department faculty and a number of universities and health-related institutions. International division faculty have worked in partnership with selected faculty and officers from each collaborating institution, exchanging knowledge and skills in health planning, epidemiology, systems improvement, medical education, community participation, health services research, and policy development.

Simultaneously, the division facilitated involvement in Latin American projects by five U.S. universities and the Association of American Medical Colleges, the American Medical Association, the Educational Council for Foreign Medical Graduates, the Education Development Center, and the Society for General Internal Medicine.

The PanAmerican Consortium for Health Policy Development

In collaboration with the PanAmerican Federation, the division has been engaged in development of the PanAmerican Consortium for Health Policy Development. Established in 1986, with support from the Pew Charitable Trusts, the consortium is a network within which schools of health professionals collaborate to address health-related social and developmental problems. The ultimate purpose of the consortium is to foster and participate in research and related activities needed to provide a basis for formulation of policies that:

- promote the development of community-based health care delivery systems throughout the Americas
- promote the development and dissemination of models of education appropriate to those systems
- foster leadership development in the region

The consortium currently consists of faculty from the following 23 health-related institutions: American Medical Association, Washington, D.C.; Association of American Medical Colleges, Washington, D.C.; Asociación Venezolana de Escuelas de Medicina (AVEFAM), Caracas, Venezuela; Asociación Venezolana para el Desarrollo de la Investigación en Salud, Caracas, Venezuela; Centro Médico Docente La Trinidad, Caracas, Venezuela; Center for Mental Healthcare Research, University of Arkansas for Medical Sciences, Little Rock, Ark; Educational Commission for Foreign Medical Graduates, Washington, D.C.; Educational Development Center, Boston, Mass.; Grupo Interdisciplinario para la Atención Primaria de la Salud (GIDAPS), Buenos Aires, Argentina; Mount Sinai School of Medicine (CUNY), NY, NY; Pan American Health Organization, Washington, D.C.; Society of General Internal Medicine, U.S.A.; University of Arizona, College of Medicine, Tucson, AZ; Universidad de Buenos Aires, Facultad de Medicina and two affiliated hospitals: Hospital Escuela José de San Martín and Hospital Privado de la Comunidad de Mar del Plata, Buenos Aires, Argentina; Universidad Católica Madre y Maestra, Facultad de Ciencias de la Salud, Santiago de los Caballeros, Dominican Republic; Universidad Centro Occidental "Lisandro Alvarado," Escuela de Medicina Pablo Acosta, Barquisimeto, Venezuela; Universidad de Concepción, Facultad de Medicina, Concepción, Chile; University of Kentucky College of Medicine, Lexington, KY; University of New Mexico, School of Medicine, Albuquerque, NM; Universidade Federal do Paraná, Curitiba, Paraná, Brazil; University of Pennsylvania School of Medicine, Philadelphia, PA; Universidad de San Carlos, Escuela de Medicina, Guatemala City, Guatemala; University of Washington, School of Medicine, Seattle, Wash.

To fulfill its goals, the consortium has conducted and promoted cooperative applied research projects, workshops, faculty development programs, technical assistance, and other complementary activities.

A critical element in maintaining collaboration among and between member institutions has been the series of interrelated workshops held in the fall of each of the first three years. The workshops addressed the question of how schools of health sciences can best contribute to the development of health systems, and in particular to the primary-care component of these systems.

The first workshop, held in 1987, was attended by an interdisciplinary group of 32 professionals drawn from the health services as well as the academic sector. Participants came from nine countries and 26 institutions. This workshop, which took place in PanAmerican Federation headquarters in Caracas, analyzed strategies employed by universities in the 1970s and 1980s and past contributions of schools of health sciences to the promotion of primary care.

The second workshop, in Caracas in 1988, was designed to promote health services research among the members of the consortium, particularly around issues related to delivery of primary-care services. Forty-five health professionals representing ten countries and 30 institutions participated in the program. The workshop considered how schools of health sciences can maximize the likelihood that results of research carried out in their institutions can and will be used to formulate health policy at the local, regional, or national level. Three primary-care projects in the process of development in Chile, Guatemala, and Venezuela were analyzed.

The third workshop, held in Antigua, Guatemala, in 1989, was organized in collaboration with the University of San Carlos de Guatemala. Thirty-eight health professionals representing 19 institutions in six countries participated in the program. The main foci were (a) the importance of evaluation as a tool for change in health services and medical education and (b) the logistic and political considerations in effective evaluation.

The consortium's activities have led to collaborative programs in six countries.

Argentina

Universidad de Buenos Aires. In 1986, the Faculty of Medicine of the University of Buenos Aires formalized a five-year agreement of cooperation with the international division. The agreement called for the department's cooperation in the development of community-based primary-health-care projects in the medical school's areas of influence. From 1986 to 1991, the university called on division staff for consultations on a variety of subjects, as well as ongoing technical assistance in the development of (a) an Adult Primary Care Program at the Hospital-Escuela José de San Martin, (b) a regional Community Health Program based at the Hospital Regional de Tigre, and (c) a manpower training program for the Hospital Privado de la Comunidad de Mar del Plata.

The Adult Primary Care Program is conducted by the Ambulatory Care Department of the Hospital-Escuela, the principal teaching hospital of the University of Buenos Aires. In many ways, the university is using the program as a laboratory to test potential innovations. The service component of the program is in its sixth operational year. Faculty from the Department of Medicine have studied the patterns of morbidity and utilization in the population served by the hospital. Results of this research are being used to restructure the organization and financing of the hospital's ambulatory care programs. The hospital is also using the program to test and promote quality assessment activities in ambulatory care. Similarly, the medical school and the Department of Medicine are using the program's education component to bring about changes in graduate education, including the introduction of training in primary-care knowledge and skills. The program has become a most highly prized rotation in the internal medicine residency.

The Regional Community Health Program in Tigre is conducted by Hospital Regional de Tigre in cooperation with the regional Secretaria de Salud. As this program enters its second year, the international division is assisting local faculty in population-based planning for the region, which has a population of 200,000.

The Manpower Training Program in Mar del Plata provides postgraduate education for faculty and residents in a group-model health maintenance organization that serves over 100,000 members. This organization is affiliated with the University of Buenos Aires School of Medicine. Since 1986, junior faculty from the University of Buenos Aires have been training in the Mount Sinai department, and department faculty have been consulting on medical education issues.

Universidad del Salvador. During 1990, the dean of the Escuela de Medicina, Universidad del Salvador, requested assistance in the development of a primary care education program.

Mount Sinai international division faculty provided planning assistance over a six-month period. The Primary Care Education Program opened in September 1991, under the direction of a former International Fellow at the Mount Sinai Department of Community Medicine.

Brazil

Universidade do Parana. The School of Medicine of the Universidade do Parana, in Curitiba, joined the PanAmerican Consortium in 1989. The university has an ambitious primary-care program in the neighboring city of Araucaria, involving the schools of nursing, dentistry, social work, and engineering as well as the School of Medicine. Since 1989 the director of the Primary Care Program has consulted frequently with Mount Sinai international division faculty. Araucaria is undergoing rapid industrialization. In consequence, the director is interested in obtaining additional training in environmental health and has recently been awarded an international faculty-development fellowship at Mount Sinai.

Chile

Universidad de Concepcion. During 1986, the dean of the University of Concepcion School of Medicine consulted Mount Sinai international division faculty on the possible reinstatement of the University of Concepcion department of preventive medicine, which had been dismantled during the military takeover of the Chilean government. International division faculty assisted the dean in 1987 in the preparation of a primary-care project proposal and remained in close communication with the university's executive officers and faculty.

Asociacion Chilena de Seguridad. In 1990, an agreement of cooperation was reached between the Asociacion Chilena de Seguridad (an employer-employee cooperative) and the Mount Sinai department's Division of Environmental Health and Occupational Medicine. The agreement calls for development of a joint research program as well as a faculty development program. Faculty development is in its first year of implementation.

Dominican Republic

Universidad Catolica Madre y Maestra. Between 1984 and 1989, the international division at Mount Sinai collaborated with the Universidad Catolica Madre y Maestra School of Health Sciences on faculty training and in the organization of the Health Services Research and Development Unit. The unit has been operating from the dean's office since 1985, providing technical assistance to the Dominican Public Health Service. The unit assisted in the organization and management of a citywide network of primary-care health centers that serve 11 geographically defined population groups in Santiago de los Caballeros, the country's second largest city.

Central Romana Inc. From 1979 to 1985, the international division assisted Central Romana Inc., part of the sugar industry, in the reorganization of a health care system operated by the company itself for a population of over 80,000. Cooperation consisted in ongoing consultations with management and with the providers of services. In keeping with the Mount Sinai division's operational principles, local leadership was identified and trained in planning, system building, program management, and principles of program evaluation. At present, the program is conducted by local administrators.

Guatemala

Universidad de San Carlos. In October 1987, the international division at Mount Sinai and the PanAmerican Consortium agreed to provide technical assistance to the Universidad de San Carlos School of Medicine in the preparation of a plan to improve health services delivery in four primary-care health centers located in separate regions of Guatemala. In addition to the development of a service program, the plan incorporated primary-care-oriented curricular changes for the medical school.

San Carlos faculty members also received technical assistance in the preparation of a proposal submitted to the W. K. Kellogg Foundation in 1990. With a fellowship awarded by the Kellogg Foundation, the director of the project received training at Mount Sinai, at the Universidad del Valle in Colombia, and at the University of Costa Rica.

Venezuela

Universidad Centro Occidental Lisandro Alvarado. During 1989 and 1990, the Mount Sinai division and the PanAmerican Federation collaborated with faculty from the Universidad Centro Occidental Lisandro Alvarado in planning a community-oriented primary-care project and in preparing a proposal to implement the project. Last year the W. K. Kellogg Foundation awarded the university a $500,000 grant to carry out the project over three years.

Educational Exchange Activities

The educational activities of the International Division of Community Medicine at Mount Sinai reflect the belief that (a) the long-term effectiveness of technical cooperation is maximal when it incorporates intensive training programs for selected local leaders, and (b) close professional relationships with local leaders are the best strategy for establishing collegial relationships with institutions abroad.

The international division is particularly concerned with the transfer and exchange of skills and attitudes needed to apply new knowledge. Since the acquisition of skills requires frequent practice, the division's programs minimize training in a traditional classroom setting and emphasize trainees' involvement in practical problem-identification and problem-solving efforts and closely supervised "learning-by-doing." Over the past eight years, the division's international graduate program has concentrated on the training of junior faculty based outside the United States. Training has taken place primarily in the trainees' home institutions and has focused on issues raised in the development and operation of specific projects in their home countries (3, 4).

From 1983 to 1991, division faculty provided formal and informal training to 21 health professionals (mostly physicians) with some experience in community medicine who wished to broaden their capabilities in the field. Nine of these individuals participated in our formal postgraduate International Fellowship. These fellows worked closely with division faculty to define an 18-month program consistent with their individual educational objectives and professional responsibilities. Each fellow designed a research or services development project that served as the practical focus of his or her fellowship activities. Most of these fellows now hold leadership positions in their own countries.

Discussion

The development of the interrelated programs and activities was made possible by the existence of an endowed chair, a committed interdisciplinary faculty team, and supplementary funding from private foundations.

Over its eight-year history, the international division has moved from its initial stage of involvement in one project, through an intermediate stage in which several institutions exchanged information on their primary-care experiences, to development and participation in a network of institutions cooperating in disseminating knowledge, improving systems, and influencing the development of health policy. Undoubtedly, the division's most important achievement to date has been establishment of the PanAmerican Consortium for Health Policy Development. International relationships established through the activities of the consortium are evolving into long-term agreements of cooperation and linkages between and among institutions from different parts of the Americas. Most important, the Latin American universities with which the consortium has worked have translated their projects into funded programs that are now being implemented, strengthening the structural/organizational framework of their institutions and enhancing capacity under the leadership of able local faculty.

The experience of Department of Community Medicine faculty who have been directly and consistently involved in the international division's activities has reinforced some home truths about technical assistance and collaboration in the international arena:

- Institutions in different parts of the world are confronting similar problems.
- All are in need of help; there are no permanent, universal experts.
- Universal solutions are elusive; the most effective solutions must be tailored to local conditions.
- A group of long-term part-time collaborators tends to be more useful than a series of one-time consultants.
- An ongoing process of faculty development and training of local professionals is more useful than the arrival of "outside consultants" who plan, manage, and/or evaluate and leave a vacuum when they depart.
- Technical support is most effective when "the outsiders" stay involved for extended periods.

In short, the past eight years have confirmed that when working at the international level, as when working in one's surrounding community, interdisciplinary teams, a participatory approach to collaboration, and continuity are critical factors for success.

The effectiveness of population-based education and research as leverage for changes that must be made by private institutions as well as government has been demonstrated. It will be used more. When population health needs are identified by community groups and made visible as sources of human risk, public and private policy is affected. This is a clear response to the challenge that Kurt Deuschle brought when he began the Department of Community Medicine at Mount Sinai. Our international role has offered a means of sharing that concern with those who in consortium are making broad-scale improvements in conditions in their own communities. In the United States as well as abroad, many children, men, and women are still without health care, decent living conditions, or opportunity to improve their lives. But the vision Dr. Deuschle provided has produced an approach and results that have gone far beyond his or our reach. He was determined to make the world a better place to live. Thanks to his vision, we and others now share that work.

References

1. Deuschle KW, Bosch SJ. Primary care and community medicine. Mt Sinai J Med 1977; 44(5):639–644.
2. Bosch SJ, Merino R, Fischer E, Deuschle KW. The international role of a U.S. medical school in developing health services. Am J Prev Med 1985; 1(4):44–49.
3. Bosch SJ, Fischer E, Freidenberg J, Merino R. Un Programa de Cooperacion Internacional que Promueve Desarrollo Social. Med Sociedad 1990; 13(5–6):25–30.
4. Bosch SJ, Casal RR, Flores MA, Insua JT, Vazquez E. La Medicina Comunitaria: Su Relacion con la Atencion Primaria de la Salud. Med Sociedad 1991; 14(3):32–37.

Kurt W. Deuschle, second from left, receiving the New York City Mayor's award for science and technology from Mayor Edward Koch, third from left, 1989. Far left, Mrs. Jeanne Deuschle; far right, George Bugiarello, chairman of the Mayor's Commission for Science and Technology.

Community Medicine:

Its Contribution to the Social Science of Medicine

Samuel W. Bloom, Ph.D., Jane Moss, Ph.D., Renate Belville, M.A., Judith Freidenberg, Ph.D.,
Debbie Indyk, Ph.D., and Edward J. Speedling, Ph.D.

Kurt W. Deuschle refers often to his experience at Many Farms, the remote Navajo reservation community where he helped to create modern front-line health care. That was in 1955, when responsibility for the health of the U.S. Indian was being transferred from the Department of the Interior to the Department of Health, Education, and Welfare. This was only the first of three major steps in his career in which he participated in significant innovations. From the Navajo-Cornell Field Health Research Project, he went on to become the first professor of community medicine at the new University of Kentucky School of Medicine. Then in 1968, he joined the original faculty of the Mount Sinai School of Medicine of the City University of New York. At each of these different steps, he was the moving force in answering the question first raised at the Navajo Reservation: "How can social scientists and members of the health sciences and professions work productively as full partners *from the beginning* of such an enterprise?" (1).

Deuschle's gradually formed answer to that question created the foundation for his pioneering conception of community medicine. It has several distinguishing features. To the traditional framework of public health medicine, he added the idea that each population targeted in clinical epidemiology must be understood as a community, with its own distinctive culture. Particularly from John Adair, his anthropologist colleague at Many Farms, he developed a deep understanding of the effects of national and ethnic culture drawn from past generations. The strategies he learned in dealing with the Navajo's way of experiencing a present that clashes with their cultural past proved adaptable to the rural communities of Kentucky. New York intrigued him for its challenge to the perspective fashioned until then in native America and rural, small-town America. In the urban ghetto surrounding Mount Sinai, he went further, following the path of urban sociologists, to understand the critical importance of subcultures in modern cities.

From these diverse situations, he found principles that were indeed universal. Respect for the client's values and institutions, which proved to be so effective with the Navajo, was equally effective elsewhere, regardless of cultural differences. An active client, working to solve his or her own health problems with the help of the professional health worker, is at less risk than one who is cast only in a passive role. These were his lifelong themes: community, culture, partnership in service.

The framework of Kurt Deuschle's career, however, was medical education. This meant a balance of teaching, research, and service based on a foundation of rigorous scientific inquiry. Therefore, the social sciences which he found so essential as he went from the Indian reservation to Kentucky, and then to the urban ghetto, were included in his vision of community medicine. Social scientists, he had learned, were specialists in the problems of communities, and he sought and won their collaboration in his life's work.

In this paper, we social scientists who have had the privilege of working with Kurt Deuschle

---

From the Department of Community Medicine, Mount Sinai School of Medicine (CUNY), New York, NY. Address reprint requests to Samuel W. Bloom, Ph.D., Box 1043, Department of Community Medicine, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

at Mount Sinai will tell the story of our collaboration. We believe it is the best testimony we can offer in his honor.

The Historical Context

In the mid-1960s, when the planning and recruitment of faculty for the Mount Sinai School of Medicine was begun, the social climate of the country was in turbulent transition. The Vietnam War, in the national memory, is usually the signal event of that period, but no less significant was Lyndon Johnson's "war on poverty." There was greatly increased funding for all services, guided by the principle of helping underprivileged persons and communities to create their own paths to a better life. Expectations among the poor rose.

In health, the goals were greater access and a single classless standard for quality of care. Medicare and Medicaid, instituted in 1965, were the major mechanisms for access. Quality was more difficult. Various professional and regulatory measures were attempted: Lawrence Weed's problem-oriented medical record is an example of the former, and the Ghetto Medicine Bill of the latter.

The Mount Sinai Hospital, in its decision to add an undergraduate medical school, was building on already distinguished academic achievements in clinical research and postgraduate medical education. The new school fit into a national movement to expand medical manpower, and substantial government resources encouraged the community-oriented medical education which Mount Sinai took as its mandate.

At the same time, the hospital itself was responding to technological changes caused by new scientific knowledge and the rapid advancement of the computer. Equally major changes in management and administration were soon to come, driven by the influx of new funding that was dependent on regulations and requirements designed to implement the social goals of access and quality. As a result, a quiet but radical revolution was occurring in the hospital, including the computerization of both the technical applications of science and the management of the social organization. Part of it was the increasing professionalization of hospital administration and changes in social structure to accommodate new fiscal reimbursement systems.

The founders of the Mount Sinai School of Medicine included in their philosophy "the importance of behavioral and environmental factors . . . in the prevention, diagnosis and management of disease" (2). They designed a program specifically to address what was identified as the "sociologic" revolution, "the third and most recent revolution" in American medical education. They wrote (2):

> Our society has expressed the desire to supplement biological excellence for the mind and dignity of the individual whether he be of the affluent or disadvantaged population. This influenced medical teaching to the point where sociology, ethics, economics and social anthropology became an essential part of medical education, though previously not considered basic to medicine. At the same time, the community became an important teaching laboratory for the medical school.

To meet these needs, they created a strong Department of Community Medicine and within it a Division of Behavioral Sciences which would assume responsibility for teaching the social sciences as part of the fundamentals of medical education.

For George James, the first dean of the Mount Sinai School of Medicine and president of the Mount Sinai Medical Center, the incorporation of social science as a partner in the solution of problems of health and illness was not new. While commissioner of health in New York City, he established one of the first social science research units to serve as an operational arm of a large government health department. At Mount Sinai, during the planning years of the School of Medicine, Dr. James established a Laboratory of Social Research, funded by program development funds from the then newly created National Center for Health Services Research. However, Dr. James was not satisfied with a limited mission for social science at Mount Sinai, totally vulnerable to the vagaries of grant support. In conjunction with the Graduate School and University Center of the City University of New York, he created a professorship in medical sociology. This position was designed to provide the leadership and a sustaining stable base for the Division of Behavioral Sciences. In July, 1968, the first appointment to this position was made and, absorbing the existing Laboratory of Social Research, the Division of Behavioral Sciences was launched. The first director to be appointed was Samuel W. Bloom. To create additional strength for the social sciences, a CUNY chair was also established in health economics, with Victor Fuchs as the first appointment.

From the outset, therefore, Mount Sinai's educational perspective included social science, and their expertise was utilized in computer-based long-term planning and in the study and facilitation of effective social organization of health services. Thus, when Kurt Deuschle arrived in 1968, he found already in place the cadre of both a social research center and behavioral science education.

To this situation so ready and eager for his approach, Dr. Deuschle added vital guiding principles. Perhaps most important was the belief, already well tested at the Navajo Reservation and Kentucky, that effective social change comes through the actions of those who have the most direct stake in the change. Rather than impose its ideas on the community, the department, under his leadership, has worked to empower community leaders by facilitating their actions to change the social conditions that cause illness. Before the term "empowerment" had the cachet it currently enjoys, Community Medicine faculty understood the meaning and the force of the idea. People put their best efforts behind change efforts they help to create.

The Division of Behavioral Science, from its inception, was composed of about six full-time sociologists, and one anthropologist was added later. Typically, the division functioned as the hub of interdisciplinary and interdepartmental activities more than as a discrete and separate unit. The research tended to be divided into distinct subgroups. One, under the direction of Jane Moss, created a team that studied the hospital as a formal organization. When applicable, research findings were implemented in collaboration with the operational staff of the hospital, and the impact of the changes was evaluated (3). Another, with Edward Speedling as its central force, functioned mainly in the Hospital Department of Human Resources, with the goal of enhancing the environment for patient care (4–5). In addition to these organized programmatic efforts, there were individual career research developments (6) and a substantial teaching contribution.

Research and Service Activities

The research and service functions of social science were, in effect, so closely coordinated that it was never simple to differentiate them. Soon after Kurt Deuschle arrived, an Office of Applied Social Research was created. Its members assumed the role of "clinical sociologists" who studied the hospital as a critical part of the "community." The hospital, which operates within a complex environment, is itself a complex organization consisting of many different constituencies and interests among employees, medical staff, and patients. How could the organization of the hospital best implement its mandates of access and quality care to the surrounding impoverished community? Concepts from the social sciences—such as structure, roles, systems—provide a way of looking at organizational problems and a way of thinking about them. But the variables for the research—schedules, priorities, visits, procedures—needed to be drawn from within the organization to produce change (7).

Related to the department's community role, Dr. Deuschle asked first that a database of the health needs of East Harlem be created. As a result, The East Harlem Community Health Study became the first major research conducted by the Division of Behavioral Science. In 1969–1970, Louise Johnson contracted with the National Center for Health services to conduct a 1,200-household probability sample survey of the East Harlem community. Of the original 1,500 households selected for interviewing, 1,163 household interviews were completed, involving a total of 3,254 people. The major objectives were stated as follows:

> The initial task which the Department had to tackle was to determine, on a scientific basis if possible, the most important health problems which this community [East Harlem] faced. It was essential the health problems be identified from the community viewpoint—that a sound, reliable and sophisticated household health study be undertaken. It was considered a departmental responsibility to help see to it that solutions to these health problems be found.

This survey provided baseline data critical for subsequent work of the department.

This was also a time of special interest in testing the efficacy of prepaid group practice as an organizing principle for more equitable quality primary care. In 1973, a grant from the Commonwealth Fund provided for a development program to study and advance the integration of service, education, and research in prepaid group practice. Through this program, close relationships were established with the Health Insurance Plan of New York for both teaching and research, conducted by both predoctoral students from the City University of New York Graduate Center and physician fellows. Focusing on the delivery of care in East Harlem, postdoctoral fellows from Latin America were included in this effort (9–11).

For two decades, the sociologists of the Office of Applied Social Research, in collaboration with the operational staff of the hospital, studied and applied their findings and evaluated the outcomes. Concurrently, they established research links with community-based organizations, which have become the focus of their present activities. For more than a decade, in the Department of Human Resources, another type of organizational development occurred that focused on staff development and patient satisfaction (12). These two research efforts are described separately below.

Studies by the Office of Applied Social Research. The Office of Applied Social Research has played a special role within The Mount Sinai Hospital, applying social science concepts and methodology to the particular problems of the hospital—problems both individual to the hospital and generic to hospitals.

In a two-year study on an inpatient surgical unit, the office studied the organization of the unit and its the barriers and options to unit-based problem-solving through ongoing qualitative and quantitative analyses. Working in partnership with operational staff responsible for the unit, the sociologists reviewed and analyzed operational problems, collected data, conferred and planned changes. Implemented changes were monitored and adjusted continuously until program objectives were met.

There were striking differences in results on problems involving systems within the jurisdiction of management on the unit, for which innovative and successful solutions were found, and problems involving centralized systems, where inability to change was the rule. Whereas hospital systems such as x-ray departments, laboratories, operating rooms, admissions, food service, and central supply operate through central planning for the hospital as a whole, individual patient care is administered in a small unit nested in a complex web of these systems. When patient care is provided, systems often overlap and disturb the priorities and routines of other systems, so no one system has a clear overall responsibility for the patient. Lack of coordination and communication between the various systems generates complaints, misperceptions, and vacuums of responsibility in overlapping system problems.

In another study, a survey combined with participant observation and qualitative interviewing, the office evaluated 12 inpatient units in which a modified unit management program had been instituted. Under this program, a core team consisting of a nursing supervisor and a physician chief, sometimes with an additional unit manager, was responsible for the management of the unit. Results from this study indicated that successful unit management was determined by the role of the physician. While the role of the nurse as team member was relatively standardized on all units, the roles of the unit chiefs were variable, resulting in discrepancies between expectations and commitments to unit management.

On units where physicians performed their primary hospital tasks on the inpatient floor, there was a relatively high level of commitment to the unit. Unit chiefs in medicine and pediatrics participated more actively in unit management because of their dependence on a well-run unit to perform their work. On units where the physicians' primary locus of performance is not on the floor but in the operating or delivery room, a more restricted level of commitment to the unit occurred. The surgeon and obstetrician are more likely to play the role of an available resource, while the actual management was left in the hands of the nurse or a floor manager. On units, such as private floors, where a large number of physicians from different services have patients, an adequate model of unit management did not seem feasible. The possibility of a unit chief who could represent the needs of so many physicians with so many different tasks was remote. In short, unit management worked best when the primary locus of performance of the physician chief was on the inpatient unit and when the unit chief had greater control over physician performance on the floor.

The office extended its program of policy research based on the constraints and options available for change among the operational staff of the hospital. One tool they developed in 1976 was an annual 10% random sample of patient data taken from newly developed hospital computer files. The sample data were merged into a hierarchical dataset accessible for analysis to answer questions relevant to hospital management and planning at relatively little cost. Use of the 10% sample for social research created a new and flexible approach to studying the organization of the hospital and describing a cross-section of patients and patient services (13).

From this database, the office produced an annual almanac of hospital services describing inpatient and outpatient demographics and utilization, including those for the East Harlem community. Semiannual reports graphed trends and quarterly changes in visits to the 156 ambulatory clinics, the major outpatient services, and the Emergency Room. This became the source of historical reference and numerous studies.

With advancing computer technology, data analysis of the total patient population replaced inferences from the 10% sample. Based on secondary analysis of hospital data, the office studied inpatient trends, characteristics of high utilizers, the workload of residents, the extent of hospital services to underinsured and uninsured patients, as well as illness and referral patterns of the hospital's inpatients and outpatients.

Hospitals operate in a constantly changing environment that continuously challenges their financial and professional viability. Throughout the country, the large teaching hospitals have responded to technological changes at a rapid pace. At the same time, they have been grappling with increasing government regulations, increased costs, and less financial support, which forced many to become more competitive to survive. These environmental factors created internal changes and pressures on the hospitals, which in turn affected their staffs and the direction of patient care services. When the inpatient reimbursement mechanism changed from per diem to per case, for instance, hospitals adopted a policy of increased turnover to maximize financial returns. The office was requested to develop a tool that would more accurately monitor the hospital's average length of stay, now a critical indicator for hospital performance. Exploratory analysis led to the design of a 12-month revolving index of length of stay, a robust instrument that accurately measured the length of stay on a timely basis, unaffected by short-term idiosyncratic fluctuations.

To study an increasingly competitive situation among hospitals, the office sociologists used material from the State Planning and Research Cooperative System, newly established to process statewide hospital discharges under the per diem system. In one study, hospitals were grouped according to their geographic proximity to other hospitals and the extent to which they shared patients from the same market area. Based on this analysis, an index of competition was established which showed that there were indeed winners and losers in this competition. Some hospitals had many empty beds; others sharing the same market were not only full but successful in refilling their beds following shorter stays.

In large measure, the research findings of the group of social scientists constituting the Office of Applied Social Research have been incorporated into the daily administrative functioning of the hospital. The focus of the group today has turned to community-based organizations. Motivating this work is the notion that applied social science must play a central role in (a) analyzing organizational barriers to care and how to eliminate them; (b) identifying ways to link research with grassroots practitioners; and (c) cultivating community-based organizations as strategic sites for integrating resources, research, services, and education.

Community-based organizations provide access to a high-risk population outside the academic center at earlier points in the disease process, with linkages to other community resources and continuity of care that is family centered. The guiding ideology of this effort is that community-based organizations and academic institutions must work in partnership so that anecdotal and observational experiences and data can be aggregated and analyzed, and the findings incorporated into practice as well as the body of knowledge of early intervention.

Patient Satisfaction and the Organizational Development Team

To enhance the environment for patient care, The Mount Sinai Hospital has developed the Patient Care Improvement Program. Based in the Department of Human Resources, it takes as its point of departure the notion that properties of the organization itself, its culture, influence behavior of the people who come to the organization both to serve and to be served. The program also assumes that optimal patient care, which occurs when patients reach their potential for well-being, can only be achieved when the men and women who provide the care act with a sense of purpose and participate in continuous efforts to improve the quality of what they do daily (14).

There is no question that this program reflects the strong influence of Kurt Deuschle's philosophy of community medicine (8). In fact, the idea of participation and empowerment is its driving force. For example, in the early 1980s when the "well-being program" began to work with service employees who had direct contact with patients, certain assumptions were made. One was that these men and women, who included building service, food service, and patient escort employees, were not in any way "ancillary" to the primary mission of the hospital. They were to be seen as making important contributions, through their daily interaction with patients and staff, to the quality of the care that patients receive at the institution. Joining the clinical, therapeutic function to work that had been considered of lesser and secondary value enabled support service employees to be approached as full partners and not as subordinates who needed to be told how to do their jobs.

Within the general well-being initiative, the first formal program was called the Patient Service Ethic Program and was offered to hundreds of employees. Small groups were organized to share their experiences. The employees did not need to be sold on the idea that their contact with patients made a real difference in patient mood, morale, confidence, and hope—the requirements for therapeutic process. They were, however, open to learning how they could be more effective. They learned by listening to each other and to the ideas put forth by the organizational development team in charge of the workshop. The team included two support service employees in addition to the social scientists. Workshops designed for nursing staff alone and for mixed groups abetted the translation of the program from seminar room to nursing floor. By request of the participants, supervisory personnel joined the workshops.

Traditional programs training employees in patient relations often begin with a set of prescribed behaviors that are presumed to have value. In this program, employees were allowed at the beginning to express their values and teach each other through the sharing of experience. Employees shaped the program that developed and were the primary agents of change because they carried the message back to their worksites.

The next program taught specific communication skills to employees. The STAR program, as it was called, built on the ground laid by the Patient Service Ethic Program. STAR is a two-day seminar that teaches win-win communication skills and how to apply them in a variety of specific situations in an acute care setting. It also teaches stress management and relaxation techniques to employees. It has subsequently been adopted as a standard feature of a number of departments of the hospital.

The organizational development team addresses system issues through a management consultation service available to all Mount Sinai managers. The consultation services include planning, conflict management, team building, and leadership training and engages individuals and groups of managers. The team works in a partnership with managers. Each party in the consultation brings some expertise in assessing the critical elements of the problem and each contributes to the solution. The result is a joint product, following a partnership rather than an "expert" model. The special achievement of partnership is that it is ongoing, building problem-solving skills that continue, and it frees the managers from the need to regularly consult experts.

The outcome of these employee development programs, of course, is intended to be more effective patient care and greater patient satisfaction. To evaluate these goals, the organizational development team, following the standards that Kurt Deuschle set for all the activities of the department, sought the most valid and reliable measures of patient satisfaction. In keeping with the partnership model, the team built a system that was accessible to managers and clinicians, allowing them to have input into the kinds of data that would be collected from patients. Currently, a quarterly survey of a random sample of Mount Sinai patients is conducted with an instrument that has been thoroughly tested with the most modern survey and statistical techniques (15).

To apply the survey results, the Patient Satisfaction Review Group has been instituted. It is an interdisciplinary body that meets after each survey and attempts to use the results to raise the quality of hospital patient services. The group is able to contribute ideas for inclusion in the survey and to request analyses to be done by the staff of the Survey Center. To increase the value of the database to the managers and clinicians who are members of the group (and who hold positions of authority in their departments), the survey data can be merged with hospital databases. Therefore, a manager can easily explore the association between levels of satisfaction and a variety of factors related to sociodemographics, the hospital experience, and the reason for admission. The system gives managers the data on which to base changes in resource allocation and the process of service delivery (16).

Another approach to data-driven change is a recent study of rounds on nursing units. This study was initiated in an attempt to engage key members of the health-care team—physicians, nurses, and social workers—in a process of strengthening rounds through understanding the factors that both foster and constrain this important activity in the hospital. A committee representing the various disciplines convened a series of "focus groups" to identify the significant issues. This, in itself, began to raise interest in the problem, but most important, these discussions were the source of a survey instrument that provided data on rounds on various inpatient units. The results have been presented to a variety of groups that have the authority to make changes to strengthen multidisciplinary rounds. Discussions to improve rounds are in process.

Consistent with the philosophy that Kurt Deuschle has epitomized, the organizational development unit has integrated contributions from both the medical and social sciences disciplines. It has succeeded in operationalizing the biopsychosocial approach (17), both in research and the delivery of medical services. Its model, constantly adjusted to the data, shows that all hospital workers, not just health professionals, have important therapeutic influence on patients. The model has provided a rationale for managers to take initiatives that give patients a greater sense of control, help allay stress, and reduce suffering. The potential effect on the hospital environment and thus on patient well-being is substantial.

Educational Activities

Kurt Deuschle's approach to medical education focused on the methodological skills of epidemiology and the critical reasoning of clinical epidemiology. In sum (6c):

> To meet society's demands for a type of medical care that is to be measured by "its impact on the health status of the population it serves," physicians must be taught to understand the denominator, the relevant population from whom the sample seen in the office is drawn. "The education doctors receive is so narrowly focused on individual case management that they have lost sight of their responsibility to the community they serve," Eisenberg argues. "Medical education must be broadened to include the concepts and methods of social epidemiology."

In other words, it is essential that medical graduates must master the rudiments of biostatistics and decision theory in order to possess "reasonable proficiency in the logic of scientific inference and the methods of statistics, [the] tools to assess the conclusions drawn in medical articles" (18).

The most sustained department contributions, therefore, were institutionalized in a first-year course in epidemiology and biostatistics and a third-year clerkship in which students conducted population-based research from problem formulation to final analysis and report.

In addition to this basic portion of the medical school's curriculum, however, the Department of Community Medicine was always involved in interdisciplinary programs. The outstanding examples are the the Introduction to Medicine and the Kellogg Blueprint for Medical Education, both interdepartmental, patient-focused teaching/learning experiences designed by Prof. Bess Dana (19).

The Division of Behavioral Sciences played important roles in all of the departmental educational activities, but also contributed distinctively on its own. Most important was a special role in liaison with the Graduate Center of the City University of New York. The centerpiece was a National Institute of Mental Health Training Program in medical sociology and community medicine established in 1969. Through this program, the division was able to function as a bridge between the CUNY Graduate Center and the Mount Sinai School of Medicine. It has served as the entry point for doctoral students in sociology, and for postdoctoral students from the fields of sociology, political science, and economics, providing them with apprenticeships in social research on medical issues. Similarly, but in lesser numbers, graduate students in psychology and anthropology have gained access to Mount Sinai through the Division of Behavioral Sciences. Moreover, faculty members from psychology, economics, and political science have found subjects for research through the good offices of this division.

These activities continue as a basic specialization within the Ph.D. program in sociology at the Graduate Center, combining in its faculty five social scientists from the Graduate School with members of the Division of Behavioral Sciences. Thirty-one students have successfully completed doctoral degrees in sociology with financial support and specialized training from the Division of Behavioral Sciences. The scientific publications resulting from this program have been substantial (20–24).

An educational model for social sciences and medicine has emerged from these activities. It is learner-centered, built on apprenticeship experience to achieve research skills, and is humanistic in its values. The increasing scarcity of federal funds for such training causes difficulties, but for several years, active operation has been sustained with resources primarily drawn from within the university.

For the future, the division has shifted its educational orientation more to postdoctoral than predoctoral training. New sources of support are being sought to maintain both Medical School and Graduate School teaching. Toward this end, planning has been initiated to create a new Graduate Center multidisciplinary program of behavioral sciences, including individuals with special qualifications in health-related research from psychology, economics, political science, social psychology, and educational psychology.

Summary

The Division of Behavioral Sciences operates today in a social climate different from that in its formative years. The federal agencies created specifically to stimulate and support social research have been cut back severely over the past two decades, and in some instances have been eliminated. The official policy of the National Institute of Mental Health, once the main source of support for both training and research in social science in medicine, has changed to a much narrower biomedical focus.

On the other hand, there are more social scientists engaged in integral roles both locally at Mount Sinai, at medical institutions nationally, and internationally (25–26). Further, reassertion of the importance for medical education of social science specifically, and of behavioral sciences more broadly defined, has been endorsed in the strongest terms by both the Association of American Medical Colleges and the National Board of Medical Examiners (27). Also, the published literature now incorporates what we in the Division of Behavioral Sciences judge to be a richer intellectual contribution to the field than ever before (28). Perhaps most important, funding for health services research and epidemiological studies has been increased in recent federal budgets. Therefore, the division is planning for expansion of both its research and education, perceiving the future as a challenge continuous with challenges of the past: finding new ways to understand the relation between social factors and the problems of health and illness.

References

1. Adair J, Deuschle KW. The people's health: medicine and anthropology in a Navajo community. New York: Appleton-Century-Crofts, 1970, 7.
2. Popper H, Nosoff R. The role of the medical school in the delivery of health care. Mt Sinai J Med 1973; 40:527–528.
3. Moss J, Belville R. Health services and social research: the bridge between. Mt Sinai J Med 1985; 52(9).
4. Morrison BJ, Rehr H, Rosenberg G, Davis S. Consumer opinion surveys. Q Rev Bull 1982.
5. Speedling EJ, Morrison B, Rehr H, Rosenberg G. Patient satisfaction surveys: closing the gap between provider and consumer. Q Rev Bull 1983; 9(8).
6. Bloom SW. Power and dissent in the medical school. NY: The Free Press, 1973.

6a. Bloom SW. Socialization for the physician's role: a review of some contributions of research to theory. In: Shapiro EC, Lowenstein LM, eds. Becoming a physician. Cambridge, Mass.: Ballinger Publ, 1979, 3–52.

6b. Bloom SW. Structure and ideology in medical education: an analysis of resistance to change. J Health Soc Behavior 1988; 29:294–306.

6c. Bloom SW. Medical education in transition: paradigm change, organizational stasis. In: Marston R, et al. Medical education in transition. New Jersey: The Robert Wood Johnson Foundation, 1992: 15–25.

7. Moss JA, Belville R. Health services research for hospital policy. Mt Sinai J Med 1985; 52:693–701.
8. Deuschle KW. Introduction. In: Johnson LA. The People of East Harlem. New York: The Commonwealth Fund, 1974.
9. Amsel B. Physicians' definitions of a prepaid group practice. A dissertation to the Graduate Faculty, CUNY, 1975.
10. Steinberg M. Consumer participation in a health care organization, the case of the Health Insurance Plan of Greater New York. A dissertation to the Graduate Faculty, CUNY, 1976.
11. Speedling EJ. Families in crisis: patterns of coping with serious illness. A dissertation to the Graduate Faculty, CUNY, 1977.
12. Morrison BJ, Rehr H, Rosenberg G, Davis S. Consumer opinion surveys. Q Rev Bull 1982; February.
13. Moss JZ, Belville R, Cornbill R. A random sample for management and planning: social research at The Mount Sinai Hospital. Mt Sinai J Med 1982; 49:479–483.
14. Speedling EJ, McDermott M, Eichhorn S, Rosenberg G. Hospital employee-patient relations: a program for enhancing patient wellbeing. Hosp Health Serv Admin 1987: Feb.
15. Speedling EJ, Nizza A, Eichhorn E, Rosenberg G, Schnepf P. The patient satisfaction review process as a pathway to quality. Mt Sinai J Med (in press).
16. Speedling EJ, Rosenberg G. Patient wellbeing: a responsibility for hospital managers. Healthcare Management Rev 1986; 11(3).
17. Engel GL. The need for a new medical model: a challenge for biomedicine. Science 1977; 196:129–136.
18. Eisenberg L. Science in medicine: too much or too little and too limited in scope. Am J Med 1988; 84:485.
19. Dana B. A proposed learning-teaching model for undergraduate medical education in comprehensive patient management. Milbank Mem Fund Q 1969; 47(4).
20. Speedling EJ. Heart attack: the family response at home and in the hospital. New York: Methuen (Tavistock), 1982.
21. Leyerle B. Moving and shaking American medicine: the structure of a socioeconomic transformation. Westport, Conn: Greenwood, 1984.
22. Bologh RW. Dialectical phenomenology. Boston: Routledge and Kegan Paul, 1979.
23. Mirotznik J, Speedling E, Stein R, Bronz C. Cardiovascular fitness program: factors associated with participation and adherence. Publ Health Rep 1985; 100(Jan–Feb).
24. Bloom SW, Zambrana RE. Trends and developments in the sociology of medicine. In: Ruffini JL, ed. Advances in medical social science. New York: Gordon and Breach, 1983, 73–122.
25. Kennedy D, Pattishall E, Baldwin DC. Medical education and the behavioral sciences. Baltimore, Md.: Association for the Behavioral Sciences and Medical Education, 1983.
26. Claus LM. The growth of a sociologic discipline: on the development of medical sociology in Europe. Leuven, Belgium: Sociological Research Institute, 1982. 2 vols.
27. Emerging perspectives on the general professional education of the physician. The AAMC Project on General Professional Education of the Physician and College Preparation for Medicine. Washington, D.C.: AAMC, 1983.
28. Bloom SW. Institutional trends in medical sociology. J Health Soc Behavior 1986; 27:265–276.

Public Health in Crisis:

The Economic Consequences of Inaction on Preventive Medicine

Marianne C. Fahs, Ph.D., M.P.H.

The tragic and threatening consequences of the fiscal epidemic of health care cutbacks in New York illustrate in miniature the profound consequences that lie ahead for the health of the nation as a whole. We are rapidly becoming a nation of big cities. Thirty years ago, less than two-thirds of the population was urban. Today, over three-fourths of the U.S. population is urban. One hundred million of our 250 million live in the largest 20 U.S. metropolitan areas (1). The challenges we face in New York are the challenges we face as an urban nation, striving to maintain a competitive edge as we look ahead to the 21st century.

The economic consequences of illness to our society are documented to be vast (1a–1d). These consequences include not only the direct medical costs of providing health care to the ill, the consequences most often cited in policy circles, but also the incalculable toll in human pain and suffering (real costs that cannot be measured), and significant, even devastating losses in economic productivity (costs that can be measured).

National Consequences

I have estimated the magnitude of these economic losses, deriving data from studies of the cost of illness by Rice et al (2). The annual loss in productivity to our national economy associated with work loss due to illness and premature death in 1991 was over $450 billion, more than 10% of total economic production (the figures for 1991 estimated by applying the prior 17-year average annual indirect cost growth rate to 1985 cost estimates, and updating the dollar values to 1991 dollars using the consumer price index). The disturbing truth is that, according to standard preventive medicine textbooks, over 90% of this economic productivity loss is preventable by current knowledge and technology.

The acquired immunodeficiency syndrome (AIDS), "the most devastating epidemic of our century," accelerates the toll of economic loss (3). Ninety-six metropolitan areas across the country with populations greater than 500,000, from Akron, Ohio, to Worcester, Massachusetts, are reporting significant caseloads of AIDS (4). Published estimates indicate the loss in economic productivity associated with the disease is especially high because it strikes young people, often at the beginning of their most productive years. The productive loss to our economy associated with the disease has been estimated at over $55.6 billion in 1991 (5).

AIDS is a national problem. However, New York is especially hard hit and shoulders a disproportionate share of this loss. Because New York State has 21% of the nation's AIDS cases, the disease is costing the New York State economy over $12 billion annually, $10 billion of that loss contributed by the toll in New York City alone (4).

A Nation of Social and Ethnic Communities: Losing Ground

"From Alexis de Tocqueville's observations about America's participation in local politics to President Bush's 'thousand points of light,' the

---

Adapted from Fahs M. The economic consequences of inaction. In: Cahill K, ed. Imminent peril: public health in the declining economy. New York: 20th Century Fund, 1991, 65-80.

From the Div. of Health Economics, Dept. of Community Medicine, and International Center for Longevity, Dept. of Geriatrics, Mount Sinai School of Medicine (CUNY), and Doctoral Program in Economics, Graduate Center (CUNY). Address reprint requests to the author at Mount Sinai Medical Center, Box 1043, One Gustave Levy Place, New York, NY 10029.

United States has long prized community activism as a source of problem solving" (6), as J. K. Inglehart notes—we are a nation of culturally diverse communities. Yet the real cutbacks in federal support of community health programs in the 1980s, and the absence of federal health policy leadership in instituting comprehensive health care reform have now shifted responsibility to state and local governments. Cities and states have been left to fend for themselves. What constituted hard times in the 1980s, during a period of economic expansion, constitutes a dangerous crisis, both fiscal and moral, in the 1990s.

The 1980s was a period of economic expansion for some, but not for others. From 1979 to 1987 the standard of living of the top fifth of the population rose by 19%. At the same time, the living standard for the poorest fifth, more than 40 million Americans, fell by 9% (7). The change is particularly striking when families with children are compared over time. In 1968, when income disparities stopped growing smaller than they had been in 1940's, the poorest one-fifth of our nation's families received 7.4% of the total income for all families; in 1987, only 4.6%. But the richest fifth increased its share from 33.8% to 43.7%. According to the Census Bureau, the income gap between the richest and the poorest is now wider than it has been at any time since the bureau began keeping such statistics in 1947.

During the 1980s, the poor became both poorer and more numerous (8); the percentage of the population living below the poverty line was:

	1975	1988
New York City	15.0%	20.8%
New York State	6.7%	8.7%
United States	12.3%	13.1%
U.S. children	16.1%	21.2%

Particularly disturbing are the children. Nationally, 1 of every 5 children lives in poverty. In New York City, the rate is 40%, double the national average; fully 60% live in families with incomes below 200% of the poverty level, compared with 43% nationally (9). Our nation's poverty rate for children is more than double the rates in countries of the Organization for Economic Cooperation and Development (10).

Increase in Poverty = Decline in Health Status. There is a direct relationship between poverty and the prevalence of serious disease (Fig. 1). Blendon and Rogers (11) found that for all six categories of chronic disease, the poor are more frequently afflicted, and for the two major categories, over twice as many poor are affected. Another study by the U.S. government (12) found that the percentage of people in the United States who say they are in only fair or poor health (as opposed to good, very good, or excellent health) rises precipitously as the income level drops: from 4.6% for people with incomes over $35,000 to 21.1% for people with incomes below $10,000. Similarly, the number of bed disability days per year is three times as high for people with incomes under $5,000 as for people with incomes above $25,000 (12).

As one would expect, with the increase in poverty among our nation's children and the real cutbacks in health and income support programs of the early 1980s, preventable childhood diseases are also on the rise, and not just in New York. Chicago's Cook County Hospital experienced a 21% increase over three years in emergency admissions for failure to thrive or diarrhea and dehydration (13). In Boston, 14% of inner-city children were found to be below the fifth percentile in growth; this is three times the expected rate (13). In Minneapolis, 20% of children from low-income families were found to have anemia (13).

A study in California found adults with chronic hypertension to have an average increase of 10 mm Hg in diastolic blood pressure following the loss of their Medi-Cal insurance benefits (14); data from the Framingham study indicate that such an increase in diastolic pressure increases a person's risk of dying by 40%. Preventable deaths among the California group of 186 working poor were documented within six months of medical termination: one from a perforated ulcer, for which the patient had delayed seeking care because of an inability to pay for it, and two from hypertensive hemorrhage and heart attack after each discontinued taking prescribed medications they could no longer afford.

The percentage of women receiving early and regular prenatal care declined significantly in the 1980s. In 1987, more than 74,000 pregnant women received no prenatal care at all—a 50% increase over the 1980 rate (15). And in the 1980s, after years of slow decline, there was an increase in the percentage of infants born with low birthweight, the most significant factor in determining infant death and disability (15).

In New York City, the situation is extreme, but it is not an isolated phenomenon. A recent report (16) identified 54 (of 353) health areas in New York City, with a total population of 650,000, in which more than twice as many deaths occurred among people under the age of 65 than the average death rates of U.S. whites as a whole predicts. In Harlem, for instance, the chief causes of "excess" mortality were cardiovascular disease (23.5% of excess deaths), cirrhosis (17.9%), homicide (14.9%), and neoplasms (12.6%). Survival analysis showed that black men in Harlem were less likely to reach the age of 65 than men in Bangladesh. Similar pockets of high "excess" mortality have been identified in other areas of the country (11–19).

Economic Consequences

What does this increase in poverty and decline in health status have to do with the nation's economy? Does not the decrease in public expenditures save public money? This reasoning is thoroughly mistaken. Radical reductions of funds have dire consequences even beyond the crippling of our ability to react positively to the many health needs of an urban environment. The short-term saving in health expenditures is, in the long run, illusionary. Economic theory can guide this analysis, but first, examine the data in four case examples, three of them concerning child health, one the health of low-income elderly women.

Example 1: Lead Poisoning

The Promise. Lead poisoning is the most common and socially devastating disease of young children, damaging the intelligence and behavior of at least 3–4 million children (20). Vulnerability to lead poisoning is associated with poverty. Because we have allowed the proportion of the nation's children living in poverty to increase from 16% to 20% over the past decade, the social factors that contribute to children's vulnerability to lead poisoning are increasing. This is an invisible epidemic that results in lifelong stupidity for its victims. It costs the country over $28 billion (20).

Public health control of this epidemic will save public money. Research shows that an investment of $32 billion in lead-poisoning-prevention programs over a 20-year period will save $60 billion in monetized returns, through averted health care costs, increased income associated with increased intelligence quotient, and averted special education costs (20). It has the additional advantage of simultaneously improving our nation's housing stock, a recommendation recently advocated by the New York Academy of Medicine (21).

The Failure. The tragedy today in New York is a lack of full funding for lead-poisoning-prevention programs. The consequences of this loss of public health leadership and vision will inevitably be borne by all of us in the future in the form of higher taxes and further losses in productivity, as we bear the double burden of the increased medical and educational costs for our affected children and find that our economic gross national product grows more slowly than it could have, owing to the disability of these individuals. The true tragedy here is that a concerted society-wide effort could virtually eliminate lead poisoning as a public health problem in this country within 20 years, and at substantial savings. In the words of Primo Levi, "When we know how to reduce the torment, but do not do it, then we become the tormentors" (22).

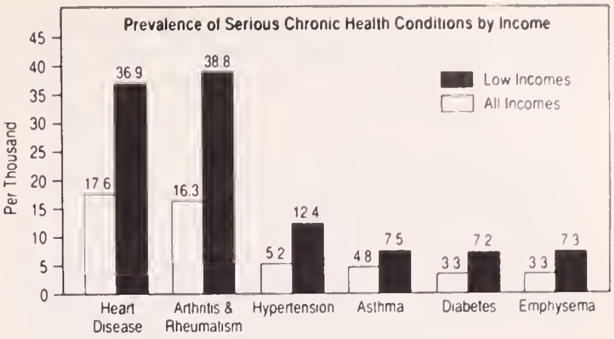


Fig. 1. Prevalence of serious chronic health conditions by income. *Reprinted with permission* from Blendon RJ, Rogers DE. Cutting medical care costs. JAMA 1983; 250:1880–1885. Copyright 1983, American Medical Association.

Example 2: Prevention of Low Birthweight

The Promise. The Office of Technology of the U.S. Congress estimates that for every low-birthweight birth averted by earlier and more frequent prenatal care, the U.S. health care system saves between $14,000 and $30,000 in newborn hospitalization, rehospitalizations in the first year, and long-term health care costs associated with low birthweight (23). This is an underestimate of the true savings to the economy because it does not include the increase in productivity we can expect when the disabilities associated with low birthweight are averted. Even with this underestimate, the Office of Technology finds that public health efforts to improve access to early prenatal care for poor women are cost saving. As the agency points out, encouraging poor women to obtain early prenatal care through expanded public health benefits is a good investment for the nation.

The Failure. As a consequence of the proposed 1992 New York City fiscal year budget cuts, all maternity services were to be eliminated:

10,000 women who were then being served, and an estimated 18,300 new clients in 1992 would not have received referrals to prenatal, postpartum, or well-child care.

Example 3: Cost-Effectiveness Of Childhood Immunization

The Promise. The cost-effectiveness of childhood vaccines is well established in the scientific literature. The first 20 years of measles vaccination provided a net savings to the U.S. economy of $5.1 billion (24). A 1983 study showed that the use of a combined measles, mumps, and rubella vaccine saved the country $60 million in direct medical costs and increased productivity (25). Another study of pertussis vaccination showed a $44 million savings in medical costs for every 1 million children vaccinated.

Despite these clearly established economic benefits, current levels of immunization in this country are less than one-half the levels in the United Kingdom, France, Canada, Israel, Spain, Italy, and Sweden (26, 27). James Mason, director of the U.S. Public Health Service, has called for an increase in the availability of immunization clinics, where approximately one-half of all childhood vaccines are delivered (28, 29). As Mason notes, "The current outbreak of measles is an ominous foreshadowing of what could be in store, if we don't make rapid progress in raising immunization coverage of preschoolers. . . . we could be laying the foundation for outbreaks of diphtheria, pertussis, polio, mumps, rubella, and congenital rubella syndrome" (29).

The Failure. As a consequence of the proposed New York City fiscal year 1992 budget cuts, over 100,000 immunizations given annually at walk-in immunization clinics in New York City would have been eliminated.

Example 4: Cervical Cancer and Low-Income Elderly Women

The Promise. A study conducted by the Department of Community Medicine at Mount Sinai of a cervical cancer screening program for elderly women attending a municipal hospital outpatient clinic in New York City showed that for every 100 Pap smears performed, the program saved over $5,000. Odd as it may seem, this study, published only 5 years ago, was the first economic analysis in this country of savings from public health prevention efforts among the low-income elderly (30).

The Failure. However, the $5.4 million proposed cut in laboratory services run by the New York City Department of Health would simply have eliminated Pap smear testing.

Although new leadership restored some of these cuts, public health productivity was substantially drained in responding to them.

Myths About the Cost Crisis

These four examples lay bare some of the economic implications of the pending public health disaster in New York City. We are mortgaging our future by systematically destroying our human and economic potential. Indeed, with the increased suffering we are imposing on our poor, we are becoming morally bankrupt as a society.

How did we as a society arrive at such a predicament? It is not as though we have not been trying to solve the interrelated and complex problems of health, productivity, and economic growth for some time. In the past few years one reform after another has been introduced, yet none has been successful. We must find new strength in the face of adversity, for the serious problems facing us are soluble. As Winston Churchill said, "Americans always do the right thing after they have exhausted all other possibilities."

After 15 years of health care reform failures under Presidents Carter, Reagan, and Bush, all based on traditional economic principles of competition and allowing the "invisible hand" of the market to rule the production and distribution of health in America, the American public is clearly ready for a major change. Poll after poll demonstrates a crisis of confidence in the American health care system (31).

The American people are disenchanted with our system of providing health care in this country, yet the policy response has focused on reduction of government expenditure. This is wrongheaded policy. It is worse than no policy at all—it is false policy. Health care expenditures are not the full economic costs, and as the case examples make clear, cuts in government health expenditures can actually increase the full economic costs the public must bear.

United States expenditures for health care are higher than any other nation's, currently 12% of gross national product, and the percentage has been steadily increasing over time (32). This is the usual statistic used to accompany the hand wringing over our current "health care cost crisis." But let us take a closer look at four superficial assumptions that health care expenditure increases should be fought by ever-more-draconian cuts to our public health care budgets.

Myth 1: Cuts in Health Care Expenditures Eliminate Health Care Services Only. A fundamental principle in economics is that expenditures equal income. In fact, health care—because it is labor intensive—creates a high rate of jobs per expenditure, much higher than, say, military expenditures, which are capital intensive. Health care employs 37 of every 1,000 Americans, compared with 28 for every 1,000 ten years ago (33). All told, the number of health-related employees has surged 43%, to 8.7 million in 1989, from 6.1 million in 1979. By the end of the 1990s, at least 12 million Americans will work in health services, according to Labor and Census Department estimates (33). Based on current trends, 7 of the 10 fastest-growing occupations in the 1990s will be in health services. Thus, despite the current slowdown in the general economy and the deceleration in employment growth, medical sector employment growth remains strong (34).

We must remain aware that cuts in public health budgets not only deny people needed medical care, they also deny people jobs.

Myth 2: Business Spending on Health Care Destroys America's "Competitiveness." It is an unproved myth that health care spending adversely affects America's "competitiveness" (35). This widely shared folklore uses as its most visible symbol the factoid that an automobile produced in Detroit now contains between $500 and $700 of health care costs. However, as Uwe Reinhardt, professor of Economics at Princeton University, has recently argued, it is not logically true that high health care costs per se (in the form of insurance premiums and worker fringe benefit packages) will render American business noncompetitive at home or abroad (35). Rather, it is the total market-clearing worker compensation package (benefits plus cash wages) that affects business competitiveness. In fact, workers have accepted tradeoffs of cash income for fringe benefits, so that total compensation packages remain competitive (35).

Myth 3: Personal Health Care Spending Increases Destroy America's "Competitiveness." It is highly unlikely that the relatively large percentage of the American gross national product devoted to health care, by itself, adversely affects the nation's competitiveness. Economists are quick to point out that there is nothing magical or necessarily wrong about the current percentage of our economy spent on health care (35, 36). In fact, the percentage could be substantially more, say 20%, without adversely affecting other productive sectors of the economy.

In 1987, Americans spent a total of $194 billion on hospital care, $102 billion on physician services, and $34 billion on drugs and sundries. These are sizable outlays. On the other hand, in the same year, Americans were willing and able to spend $35.6 billion on tobacco, $61 billion on alcohol, $24.2 billion on jewelry, and $26.2 billion on cosmetics (35)—none of which increase productivity. Arguments that spending in health care is a misallocation of productive resources must contend with these economic statistics.

Myth 4: Government Spending on Health Care Destroys America's "Competitiveness." The only way in which high health expenditures are likely to detract from the nation's competitiveness in the long run is if public health care spending comes at the expense of our investment in other forms of human capital, such as education, or the nation's infrastructure, both of which are largely publicly financed (35). And this would only happen if American taxpayers and their political representatives insist on keeping the percentage of the gross national product going through public budgets constant, so that more spent on public health care (about 41% of all health expenditures at the present time) means less spent on other public programs (35).

Our tax burden in this country (33% of the gross national product) remains lower than that of all the European nations in the Organization for Economic Cooperation and Development (OECD), where tax-to-GNP ratios range in the mid-to-high 40% range (37). We remain the lowest, despite a growing underclass of poverty-stricken children, an increase in the elderly population, and higher infant mortality rates and lower life expectancies than in almost all other OECD countries (38).

Research shows a strong positive correlation (Pearson *r* 0.635) between life expectancy and public health expenditures as a percentage of gross national product (38). Similarly, countries giving high priority to health programs have infant mortality and child death rates approximately 2–3 times lower than their counterparts at the same level of economic development (39). These correlations do not hold for private health expenditures. Yet the United States has the lowest ratio of public-health to total-health expenditure of all 24 OECD countries, which at 41% is comparable only to the ratio in Turkey (40). Other countries average between 70% and 80%. This higher public health allocation does not adversely affect their GNP growth. Compared with Canada and Japan, our average annual GNP growth per capita between 1985 and 1988 in real dollars ranks a poor third. Japan was first at 3.6, Canada second at 3.0, and the United States third at 2.6 (41).

In New York City, the public health care budget did not keep pace with the growth in real GNP during the 1980s and has been continually losing ground in real dollars. However, the substantial restrictions of the proposed 1992 New York City budget mark the first reversal in public health expenditures. The impact of these shrinking resources will clearly adversely affect the city's health. Econometric analyses have shown a quantifiable direct relationship between health expenditures and age-adjusted mortality (42). Based on these equations, we can expect the age-adjusted mortality in New York City to increase.

Reality of the Cost Crisis: The Consequences of Competition

We are all "the slaves of some defunct economist" as John Maynard Keynes pointedly noted. The current federal government policies of inaction are based on the economic theory of competition, which embodies the principle of laissez-faire—literally letting the private market alone to do its work. The Reagan and Bush administrations have used the theoretical ideal of competition and "privatization" to sound the alarm against rising public health care expenditures. This alarm is combined illogically with the reassurance that cuts in public programs and stiffer regulation of hospitals and physicians will solve the cost problem.

Competition used this way is a wolf in sheep's clothing. The theory, as first detailed by Adam Smith in his classic *The Wealth of Nations,* predicts that the ultimate outcome of the God-like "invisible hand" guiding the private market will be compatible with both private profit-seeking and the public benefit. However, even Adam Smith had moral qualms about this compatibility. According to Smith, the interest of those who live by profit "is never exactly the same as that of the public" and they "have generally an interest to deceive and even to oppress the public, and . . . accordingly have, upon many occasions, both deceived and oppressed it" (43).

Moral qualms aside, the economic and health policy literature is full of reasoned arguments and empirical proof that the special quality of health care does not meet the stringent requirements necessary before the ideals of competition can be approached (44–47). For example, consumers must have time to search the market to exercise their sovereignty, yet the need for medical care is often urgent, leaving the consumer no time to shop for the best price. Would-be physicians and other health professionals must have free entry into the market, which goes absolutely against the standards of the American Medical Association and state licensing boards. And consumers must have perfect knowledge about the quality of the product produced by individual caregivers and medical institutions, which is extremely difficult to attain in the complex and constantly changing field of medicine.

Let us examine four consequences of the past decade of "competition" in health care.

Consequence 1: Higher Costs. National health care spending has more than tripled from $248 billion in 1980 to over $800 billion this year—a spending increase of over $1,000 per person. Adjusted for inflation, health care expenditures have been growing at near record levels (34).

Consequence 2: Increased Regulation and Malpractice Suits. The "market"-oriented policies of the Reagan and Bush administrations have left us with an erosion of clinical freedom. Physicians in the United States are now the most litigated-against, second-guessed, and paperwork-laden physicians in Western industrialized democracies. Rates of malpractice suits against physicians in private practice more than tripled between 1980 and 1985 (48).

Consequence 3: Loss of Health Insurance for Americans Who Need It Most. Because of the rising cost of health insurance premiums, insurance companies, including even Blue Cross, have responded to competition by dropping "bad risks." The horrible irony is that these are the people who are most at risk for disease and disability and need coverage most. The numbers of uninsured have increased from 30 million in 1980 to approximately 37 million in 1987, and millions more are underinsured (49).

Furthermore, the proportion of children in the United States without private or public health insurance increased from about 13% in 1977 to 18% in 1987, according to a 1990 study by the Agency for Health Care Policy Research (49) (Fig. 2). In 1991, one-third of all poor and low-income children have no health insurance, a consequence of the decrease in private coverage among low-income families and the decrease in public coverage of single-parent families (49). Again, the tragedy is that these are children with the highest health needs. Among the poor and near-poor, there is an increased incidence of a range of diseases, including respiratory infections, tuberculosis, measles, dysentery, childhood lead poisoning, and AIDS. Perpetuation of the cycle of poverty is a high risk among these children.

Consequence 4: Deteriorating Administrative Efficiency. In the United States, many different health insurance companies compete with each other. For this reason, the proportion of health care expenditures consumed by administration in the United States was 60% higher than in Canada and 97% higher than in Britain in 1983 (50). Administrative costs in the United States increased 37% in real dollars between 1983 and 1987, whereas in Canada they declined. The proportion of health care spending consumed by administration is now at least 117% higher in the United States than in Canada (50).

Absence of Political Will to Promote Public Health: The Consequences

The economic consequences of inaction on public health needs are severe. They include higher direct expenditures on medical care for the costs of disease that could have been prevented, and decreases in worker productivity. It is an economic truism that investment in capital increases labor productivity. Our neglect of human capital investment will surely decrease our rate of GNP increase; health is a crucial contributor to economic growth.

Health care spending is a productive use of resources. We must educate the American public to understand what a payoff the right kind of public spending can have. If we lose the productivity of our poor and near-poor, we lose economically; but we also lose a profound American dream, that is, the dream of betterment of the poor or working-class immigrants who work hard so that their children can go to college. The loss of access to health care during the 1980s is already robbing many Americans and their children of an opportunity for a long and healthy life. A young uninsured adult man now is three times more likely to die during a hospitalization than an insured male patient similar in every way except for insurance status (51). The death of this man is a preventable tragedy and can mean the end of the American dream for his young children, who must attempt the struggle toward economic improvement without the financial support of their father.

The possibility that we will achieve the economic expansion that we are potentially capable of will be met only by an active public health policy agenda, with target objectives and target budgets clearly spelled out. Without a positive federal economic and political agenda for the health of the nation, we are in danger of lurching off track every time political pressures present unexpected choices.

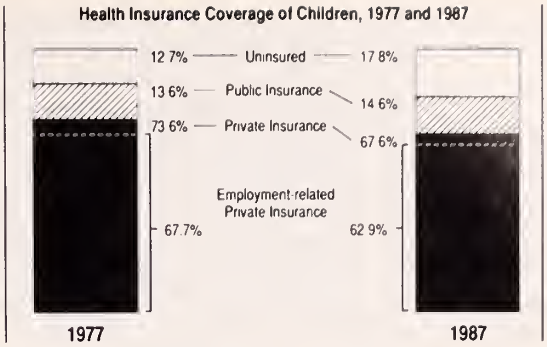


Fig. 2. Health insurance coverage of children, 1977 and 1987. *Reprinted with permission* from Cunningham PJ, Monheit AC. Insuring the children: a decade of change. Health Affairs 1990; 9(4):76–90.

Options for the Future

Government can simply look the other way, or we can summon the courage to face the problem squarely. Our economic growth in the future depends on investment in public health today.

Reduce Poverty. A good investment choice is to reduce the number of Americans living in poverty. One way to approach this problem efficiently without increasing taxes is to decrease the income disparity through a negative income tax policy. This policy would redistribute the tax burden more equitably among income brackets, the lowest brackets getting cash returns. To quote one of the great leaders in the history of public health, Johann Peter Frank, "Poverty is the chief cause of disease" (52).

Rebuild Community Health Centers with Federal Funds. Another investment choice is to form a federal-community partnership and rebuild our public health infrastructure to improve access. The decrease in funding that community health centers have suffered has had a devastating impact on the poor served by these programs. This has been a myopic cost-cutting policy. Evaluations of these centers have found that these programs are a cost-effective use of federal health expenditures. They are targeted to high-need populations and have been highly successful in reducing hospitalization rates of patients served. Reductions in hospitalization rates among center users range from 25% to 67% (53–55).

Establish Single-Payer System. How could a federal-community partnership be financed? One way is to make sure that all community health center clients are already insured when they arrive at the health center. We have the capability to achieve that at the present time, with no additional taxes. The General Accounting Office has reported that a single-payer system for medical care similar to that in Canada would yield annual savings of $75 billion. This is more than enough to cover all the uninsured, with enough left over to cover all the deductibles, copayments, and out-of-pocket costs for medical care to all others who are now insured (56).

Find New Investment Capital through Excise Taxes that Would Cut Deaths. However, to rebuild our public health infrastructure and increase the availability and accessibility of health services to our disadvantaged communities, we will need new creative and culturally sensitive community programs and outreach efforts, as well as new investment capital for these community efforts. We can have higher taxes, or we can have different taxes.

We might examine the use of excise taxes by the federal government, particularly excise taxes on alcohol and cigarettes, which have been relatively stable since 1951 (57). Economic policy research shows that if we were to raise the excise tax on beer in line with the rate of inflation over the last three decades, we would cut motor vehicle fatalities, many of them alcohol related, among 18- to 20-year-olds by about 15%, saving more than 1,000 lives per year. Moreover, if we were to restore the excise tax on cigarettes to its real 1951 value, 800,000 premature deaths in the cohort of Americans 12 years and older would be averted. Such excise taxes can be considered public health investment taxes and, if used to improve services to the poor, will have a public-health multiplier effect on national health levels and economic wealth.

Focus on Preventive Medicine

We remain a nation of communities. Some of our communities are desperately ill. We cannot ignore them; the cost is too high. We must fight against ignorance, political expediency, and selfishness to uphold the ideals of community medicine, first advanced a generation ago by Kurt Deuschle, the founder of community medicine in the United States. As he has said, “In the final analysis, the diagnosis and treatment of community and social pathology, not individual pathology, must be our major concern” (58).

Our public health programs are among the most cost-effective health programs in the country. Action must come soon, or health care problems will bring this country to its knees. The most rapid and dramatic improvements in the health of the public will result not from technology-intensive medical care, but from preventive measures. These are now grossly underfunded; only 2.9% of all health dollars is spent for government public health activities. We must turn away from narrow-minded cost-containment refrains to consider the health of our country. We need rational analysis, compassion, and an appreciation for the long-run, as well as the short-run, aspects of this complex problem.

Joseph Califano, former Secretary of the Department of Health Education and Welfare, wrote in his autobiography:

> Of course, those who govern will make mistakes. Plenty of them. But we should not fear failure. What we should fear above all is the judgment of God and the judgment of history if we, the most affluent people on earth, free to choose and act as we wish, choose not to govern justly, choose not to distribute our riches fairly and to help the most vulnerable among us, or worse yet, choose not to even try.

Acknowledgments

I thank Kurt Deuschle, M.D., Philip Landrigan, M.D., M.Sc., Charlotte Muller, Ph.D., Alan Silver, M.D., Ellen Fisher, Ph.D., M.P.P., and Ray Cornbill, M.B.A., for helpful comments and insightful criticisms of earlier drafts; and Ruth Labovitz, B.A., for expert research assistance and Brett Penney, B.A., for patient technical assistance.

References

1. U.S. Bureau of the Census, U.S. census and population; 1960 and 1970; 1980 census of population, vol. 1, chapter A (PC80-1-A) and Supplementary Report, Metropolitan Statistical Areas (PC80-81S1-18); Current Population Reports, Series P-26, No. 85-AL-C to 85-WY-C and Series P-25, No. 1039.
1a. Rice DP, Hodgson TA, Kopstein AN. The economic costs of illness: a replication and update. Health Care Financing Rev 1985; 7(1):61–80.
1b. Scitovsky AA. Estimating the direct costs of illness. Milbank Mem Fund Q 1982; 60(3):463–491.
1c. Hodgson TA, Meiners MR. Cost-of-illness methodology: a guide to current practices and procedures. Milbank Mem Fund Q 1982; 60(3):429–462.
1d. Salkever DS. Morbidity costs: national estimates and economic determinants. Department of Health and Human Services Publication No. (PHS) 86-3393. Washington, D.C.: National Center for Health Services Research and Health Care Technology Assessment, October 1985.
2. Rice DP, Hodgson Ta, Kopstein AN. The economic costs of illness: a replication and update. Health Care Financ Rev 1985; 7(1):61–80.
3. Osborne JE. AIDS and public policy. AIDS 1989; 3(Suppl 1):S297–300.
4. Centers for Disease Control. HIV/AIDS surveillance report. U.S. Department of Health and Human Services, July 1991, 1–18.
5. Scitovsky AA. The economic impact of AIDS. Health Affairs 1988; 7(4):32–45.

6. Inglehart JK. From the editor. Health Affairs 1988; 7(4):4.
7. Passell P. Forces in society, and Reaganism, helped dig deeper hole for poor. New York Times July 16, 1989.
8. Office of Policy and Financial Management. Human Resources Administration, the City of New York. July 1991. Unpublished data.
9. U.S. Bureau of the Census. Current population survey for NYC, average of 1985–86 estimates of percent of population living in poverty. National data from Current Population Report. Series P-60, #158.
10. Smeeding T, Torrey BB, Rein M. Patterns of income of poverty: the economic status of children and the elderly in eight countries. In: Palmer JL, Smeeding T, Torrey BB, eds. The vulnerable. Washington, D.C.: The Urban Institute Press, 1988:89–119.
11. Blendon RJ, Rogers DE. Cutting medical care costs. JAMA 1983; 250(14):1880–1885.
12. Health status of the disadvantaged, Chartbook 1986. DHHS Publication No. (HRSA) HRS-P-DV86-2. Washington, D.C.: U.S. Government Printing Office, 1986.
13. Mundinger MO. Health service funding cuts and the declining health of the poor. N Engl J Med 1985; 313(1): 44–47.
14. Lurie N, Ward NB, Shapiro MF, Brook RH. Termination from Medi-Cal—does it affect health? N Engl J Med 1984; 311:480–484.
15. The National Commission to Prevent Infant Mortality. The Honorable Lawton Chiles, Chairman. Troubling trends: the health of America's next generation. Feb 1990; 330 C St. SW, Rm. 2006, Washington, D.C.
16. McCord C, Freeman HP. Excess mortality in Harlem. N Engl J Med 1990; 322(3):173–177.
17. Jenkins CD, Tuthill RW, Tannenbaum SI, Kirby CR. Zones of excess mortality in Massachusetts. N Engl J Med 1977; 296:1354–1356.
18. Manton KG, Patrick CH, Johnson KW. Health differentials between blacks and whites: recent trends in mortality and morbidity. Milbank Q 1987; 65(Suppl 1):125–199.
19. Kitagawa EM, Hauser PM. Differential mortality in the United States: a study in socioeconomic epidemiology. Cambridge, MA: Harvard University Press, 1973.
20. U.S. Department of Health and Human Services, Centers for Disease Control. Strategic plan for the elimination of childhood lead poisoning. Washington, D.C., February 1991.
21. Landrigan PJ. Housing and health: conclusions and challenges for the future. Bull NY Acad Med 1990; 66(5): 587–591.
22. Levi P. The periodic table, 1st American ed. New York: Schocken Books, 1984.
23. U.S. Congress, Office of Technology Assessment. Healthy children: investing in the future. OTA-H-345, Washington, D.C.: U.S. Government Printing Office, February 1988.
24. Bloch AB, Orenstein WA, Stetler HC, et al. Health impact of measles vaccination in the United States. Pediatrics 1985; 76(4):524–532.
25. White CC, Koplan JP, Orenstein WA. Benefits, risks and costs of immunization for measles, mumps and rubella. Am J Public Health 1985; 75(7):739–744.
26. UNICEF. The state of the world's children. New York: 1987.
27. World Health Organization. 1986 world health statistics annual. Geneva: 1986.
28. U.S. Congress, Office of Technology Assessment. The implications of cost-effectiveness analysis of medical technology. OTA-H-126. Washington, D.C.: U.S. Government Printing Office, August 1980.
29. Mason J. The nation's health. Official Newsletter of the American Public Health Association. August 1991.
30. Mandelblatt JS, Fahs MC. The cost-effectiveness of cervical cancer screening for low-income elderly women. JAMA 1988; 259(16):2409–2413.
31. Blendon RJ, Donelan K. Interpreting public opinion surveys. Health Affairs. Summer 1991; 10(2):166–169.
32. Health Care Financing Administration, Division of National Cost Estimates, U.S. Department of Health and Human Services, 1989.
33. Freudenheim M. Job growth in health care soars. New York Times March 5, 1990.
34. Levit KR, Lazenby HC, Letsch SW, Cowan CA. National health care spending, 1989. Health Affairs. Spring 1991; 10(1):106–116.
35. Reinhardt UE. Health care spending and American competitiveness. Health Affairs. Winter 1989; 8(4):5–21.
36. Fuchs VR. The health sector's share of the gross national product. Science 1990; 246:534–538.
37. Organization for Economic Cooperation and Development Secretariat. Health care expenditure and other data. Health care financing review. 1989 annual supplement, Baltimore, MD: U.S. Department of Health and Human Services, December 1989.
38. Roemer MI, Roemer R. Global health, national development and the role of government. Am J Public Health 1990; 80(10):1188–1192.
39. Cereseto S, Waitzkin H. Economic development, political economic system and the physical quality of life. J Public Health Policy Spring 1988; 104–120.
40. Schieber GJ, Poullier J-P. International health spending: issues and trends. Health Affairs. Spring 1991; 10(1): 106–116.
41. U.S. Department of State, Bureau of Intelligence and Research. Economic growth of IECD countries, 1978–1988. Report No. IRR 205 (revised), 1989, and unpublished data.
42. Hadley J. More medical care, better health? Washington, D.C.: Urban Institute Press, 1982.
43. Heilbroner R. Economic predictions. New Yorker July 8, 1991.
44. Hadley J, Holahan J, Scanlon W. Can fee-for-service reimbursement co-exist with demand creation? Inquiry 1979; 16:247.
45. Rice T. The impact of changing Medicare reimbursement rates on physician-induced demand. Med Care 1983; 21: 803.
46. Rossiter LF, Wilensky GR. A re-examination of the use of physician services: the role of the physician-initiated demand. Inquiry 1983; 162.
47. Fahs MC. Physician response to the United Mine Workers cost sharing program: the other side of the coin. Health Serv Res (in press).
48. Lee PR, Etheredge L. Clinical freedom: two lessons for the UK from US experience with privatisation of health care. Lancet Feb 4, 1989; 263–265.
49. Cunningham PJ, Monheit AC. Insuring the children: a decade of change. Health Affairs 1990; 9(4):76–90.
50. Woolhandler S, Himmelstein DU. The deteriorating administrative efficiency of the U.S. health care system. N Engl J Med 1991; 324(18):1253–1258.
51. Hadley J, Steinberg EP, Feder J. Comparison of unin-

sured and privately insured hospital patients: condition on admission, resource use and outcome. JAMA 1991; 265(3):374–379.

52. Sigerist HE. Medicine and human welfare. College Park, MD: McGrath, 1970.
53. Davis K. Primary health care: past, present and future. Paper presented at the Columbia University School of Social Work, National Interdisciplinary Conference on Primary Health Care. Arden House, Harriman, NY, May 30–31, 1981.
54. Freeman HE, Goetsch G. Use of community health centers: summary tables. Institute for Social Science Research, University of California, Los Angeles, September 1980.
55. Davis K. Expanded use of community health centers: potential savings to Medicaid. Paper prepared for 1981 Commonwealth Fund Forum, Medical care for the poor: what can states do in the 1980's? Philadelphia, PA, Aug 9–12, 1981.
56. U.S. General Accounting Office. Report to the chairman, Committee on Government Operations, House of Representatives. Canadian health insurance: lessons for the United States. GAO/HDR-91-90. June 1991.
57. Grossman M. Health benefits of increases in alcohol and cigarette taxes. National Bureau of Economic Research, Reprint No. 1414. Cambridge, MA.
58. Deuschle KW, Eberson F. Community medicine come of age. J Med Educ 1968; 48(12):1229–1237.

Community Partnerships in the 1990s:

Can Urban Medical Centers Meet the Challenge?

Gary Rosenberg, Ph.D., Helen Rehr, D.S.W., and Susan Blumenfield, D.S.W.

No institution is separate from its times (1), nor can individual professions, programs, and services be separate from their times. In the 1990s, reeling under divergent pressures, particularly economic, hospitals are reassessing their roles, the services they provide, and the manner in which they provide them to the community. To emerge from such reassessment as a major positive force in the community, a hospital must rediscover its traditional mission and role vis-à-vis the community, expand its boundaries to embrace the broader community, create innovative partnerships with its various communities, and develop effective solutions to community problems (2).

This charge to hospitals may be a particular challenge to urban academic institutions, whose roles are steeped in clinical, research, and educational imperatives, and whose surrounding communities are plagued by acute biopsychosocial and economic problems of overwhelming magnitude. Balancing traditional imperatives and issues of financial survival with community needs is no small task.

To place in context the urban medical center and the challenges of the 1990s, a brief overview of the times and forces that influence the urban medical center is of value. We will then examine some of the programs, services, and philosophies of service that are products of those times and that may form the foundation for meeting the challenges and opportunities ahead.

The Urban Medical Center In Context

Each decade of this century, with its attendant social, economic, and health issues, has had a major impact on medicine and health care and on hospitals. No one article can presume to do more than highlight these phenomena and their impact on the urban medical center. From the vantage of retrospection it becomes apparent that basic social-health ills remain unchanged in the 1990s from conditions at the beginning of the 20th century; they are, in fact, magnified. In urban environments, infectious and chronic disease, poverty, unemployment, and issues of inaccessibility and fragmentation have been constant. Added are the new problems of drug abuse, crime and violence, homelessness, and AIDS, the epidemic of the 1980s. Limited resources and mushrooming demands on those resources further complicate, if not jeopardize, solutions to social-health problems. These are legacies of the times we can well do without. They create in our inner cities a veritable caldron of potential storm, whose eruption we have witnessed only recently in Los Angeles and whose aftermath further drains scarce financial resources.

While basic social-health ills have seemingly remained unchanged, the health care institution itself has changed, owing to advances in medical and information technologies, specialization in diagnostic and treatment services, the proliferation of professions and paraprofessions, the advancement of professional practice, a greater concentration on ethical issues in health care, participation by and greater knowledge and awareness of consumers, and changes, in some institutions, in leadership values and emphasis.

The urban medical center of the 1990s is a far cry from its turn-of-the-century predecessor, typ-

---

From the Department of Community Medicine (Social Work), Mount Sinai School of Medicine (CUNY), New York, New York. Address reprint requests to Gary Rosenberg, Ph.D., Edith J. Baerwald Professor of Community Medicine (Social Work), Box 1246, Mt. Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

ically a charitable institution for the needy that reflected the idiosyncratic qualities of the community it had originated to serve. These early institutions were seen as community institutions in that "they usually served a population defined in geographic, ethnic, or cultural terms and were both supported and held accountable by the leaders of their communities" (3). The urban medical center is at once the product of such origins and of forces in its various times, which place it in dynamic tension with its various communities. The forces of the past 30 years have been the most influential in shaping the urban medical center of today and in creating the tensions that exist between the medical center and its various internal and external communities, despite existing partnerships with such communities.

From the Sixties to the Present. Out of the 1960s, which were characterized by the liberal tenets of the Great Society, came the health care policies and seemingly unlimited funds and entitlements that fostered establishment of numerous community programs and projects and a surge in technologic advancements. Many of today's partnerships between the urban medical center and its communities are by-products of the various community programs and projects spawned in this era. Many were natural by-products; others were the consequence of a growing involvement of federal government in financing research, construction, and medical education, which changed the relationship between institutions and their local communities.

By the 1970s, the well of seemingly unlimited funds was beginning to run dry and the "three Cs" emerged—control, caps, and cutbacks. Regulation in the form of utilization review forced hospitals to examine the need for specific medical services and length of stay. Throughout the 1970s and the 1980s, hospitals and health care professionals were also responding to demands for increased accountability for the quality of care, in the form of both private and government-mandated programs (JCAHO, PSROs, and PROs) and their own professional organizations. As health care costs spiraled, the government, business and industry, and third-party payors added pressure to contain costs while maintaining quality, for example with the Medicare prospective payment plan based on diagnosis-related groups. Each of these activities and requirements placed hospitals at odds with cost-containment efforts as small industries grew around the efforts and new staff and technical and other resources were necessary to implement imposed requirements.

During the 1980s, health care institutions entered an era of aggressive competition, became commercialized, and adopted the unfamiliar language, practice, and techniques of business, requiring a new dimension of expertise among the leadership. As we entered the decade of the 1990s, hospitals faced issues such as physician payment reform through use of a resource-based relative value scale, the Medicare capital payment fold-in regulation, the push for health care reform, increased media attention to health care policy and financing and delivery issues, increased demands from community organizations for expanded roles within the medical center, and increased demands from patients' employers for cost-effective care.

Today, fiscal concerns appear to dominate, and the business imperatives of the urban medical center seem, to many, to override its community service mission.

The Urban Medical Center of Today. In the face of these legacies of the past and forces of previous and present times, the urban medical center of the 1990s is experiencing a tug-of-war between its various roles, numerous internal and external pressures, and the fundamental imperative to provide quality patient care, but within limited financial resources. By its nature a sophisticated, resource-intensive, highly specialized organ, the urban medical center often is perceived by the inner-city community as an elitist monolith whose objectives have little to do with the needs of the surrounding community. A microcosm of society, the urban medical center demonstrates all the strengths and weaknesses of society, many of the weaknesses erecting barriers to meeting the challenges in present times, particularly barriers to creating new or enhancing existing partnerships with the various communities it comprises and serves.

Barriers to Effective Partnerships

If barriers to effective partnerships exist within an urban medical center, what are they and how can they be overcome?

Definition of Partnership. Perhaps the first barrier to identify and overcome is that of definition. Dismissing for the moment the legal aspects of partnerships, of which there are some within the institution, in 1990s parlance a partnership is a strategic alliance involving a close relationship between parties that have specified joint rights and responsibilities. A partnership by its nature involves a degree of tension, as each of the parties to the partnership seeks to ensure its rights and often, in anxiety about rights and needs, ignores responsibilities. This tension and consequent focus on rights is a barrier to achieving those rights, unless the definition also includes agreement regarding the central, shared purpose and focus of the partnership, thereby making clear each partner's responsibilities. With this in mind, it is clear that the urban medical center faces a number of dilemmas in any effort to define and/or create partnerships with its various communities, particularly within the immediately surrounding, external community and its community-based organizations.

The first dilemma surfaces when one attempts to reach agreement regarding the central, shared purpose and focus of the partnership. While it may be politic to cite the shared purpose and central focus as "the individual and his or her right to care and right to quality care," in reality, both the medical center and community-based organizations have a set of business, organizational, and financial imperatives that put them in conflict with their own basic missions of community service and that often place them in competition with one another for scarce resources.

A second dilemma stems from a lack of homogeneity within and among existing community groups, organizations, and coalitions, caused by diversity of community problems and needs as well as diversity of the population within a given community. This may exacerbate the tension in one or more individual partnerships with the medical center, because the definition of responsibilities may differ in each group, but the call for individual rights is no less demanding.

A third dilemma is in the emphasis on and the order of priority of issues. While the urban medical center focuses on issues related to the provision of health care, community priorities often start with housing, employment, police protection, and traffic control before focusing on health care. What is intriguing about this dilemma, however, is that it brings us full circle: to the recognition that we define all our communities differently, that both the medical center and all its communities have more than a single mission, that each new partnership is a unique negotiation of rights and responsibilities for both potential partners, and that each existing partnership needs to be reassessed and renegotiated in the wake of dynamic forces within society that continually affect both the community and the medical center.

Territorialism and Control. These barriers are the by-products of a competitive society. We compete for recognition for ourselves and our departments, often "rewriting history" in the quest for such recognition. We compete for funds to protect or sustain our own programs or pet projects, sometimes to the unfortunate detriment of another critical program or project. We protect our own personal sources of income and advancement to the detriment of others. We foster fragmented activities or thwart progress toward effective planning in order to control the balance of power within a system. The politics of the system takes on a life of its own, and whoever plays the politics cleverly broadsides the political neophytes.

Somewhere, while we are all protecting our own turf, whether it be internal or external to the institution, the raison d'être of our existence—the patient and the community—is lost.

Lack of Communication. Communication barriers occur in many forms, ranging from lack of effective communication and agreement within the institution regarding mission, purpose, and goals and objectives to an inability to listen to and hear what the internal and external communities are trying to communicate. Although many organizational groups and a plethora of print media exist, they may not be effective if the information from the groups is not being acted on and the message in the media are not absorbed. The hospital must have a pipeline to the community. Moreover, although the number of groups, coalitions, boards, and committees may be great and their representation of group voices may be significant, effective communication may not exist if other barriers, such as "turfism" and lack of clarity, intervene.

In addition, barriers to effective partnerships will continue unless there is vigorous leadership at critical levels within and outside the institution, inculcating a value system and culture that support effective internal and external partnerships. Barriers will also remain unless there is systematic, integrated strategic planning, in which every action, every new program, every new investment fits the future that the hospital and its community have defined.

Barriers will also exist if the medical center perpetuates or does not counteract the community's perception of it as an elitist monolith that has forgotten its origins. For the urban medical center to renew its origins in, philosophy of, and commitment to community service, it must examine its existing programs, services, departments, and internal and external partnerships. It will most likely find that it has already built a foundation on which it can enhance existing community partnerships and build innovative new programs for the future.

The Mount Sinai Medical Center: Building a Foundation for Community Partnerships

The Mount Sinai Medical Center, a 136-year-old tertiary-care center in New York City, is representative of academic institutions in urban settings. Located in the heart of East Harlem, Mount Sinai has experienced the tug-of-war between the traditional roles of an urban medical center and the pressing needs of a poor, medically underserved, underinsured, even uninsured population. East Harlem has a population of 110,508, or 7.4% of the total population of Manhattan. Residents are predominantly low-income first- and second-generation Hispanic (52%) and African-American (39%). Approximately 70% of households have incomes of less than $15,000; 35% receive public assistance. In virtually all measures of health status—including mortality, infant mortality, teen pregnancy, birthweight, prenatal care, and infectious disease—East Harlem compares unfavorably at local, state, and national levels (4).

While Mount Sinai is a product of its times and social forces, it is also the product of its Jewish founders' commitment to social philanthropy and of those in its 136-year history who championed a social-service orientation.

Origins in Social Service. Mount Sinai had its origins in 1855 as a custodial home for the needy. It was a social institution in which medicine played but a small part. When the home became incorporated as a voluntary, not-for-profit community hospital in 1866, it began to take on more of a medical complexion, but did not lose its social orientation. Social services were performed by women auxiliaries visiting patients in the hospital and at home, focusing on social problems precipitated by illness. Their value and ongoing commitment was formally recognized in 1916 in the creation of the Auxiliary Board, whose objective was to "supplement medical care provided by the hospital with services [that] further the welfare of the patient and family" (5). The Auxiliary Board remains a vital, visible, and effective partnership within the medical center.

Creation of a Social Work Department. Earlier, in 1906, as the result of efforts of Mount Sinai medical and lay leaders, a Department of Social Welfare, one of the first in the country, was created and financed. Throughout its 86-year history, what is now the Department of Social Work Services and its leaders has striven to integrate social work activities into the mainstream of patient care services and to establish partnerships with the community and various community organizations.

Social work leaders initiated strong mutual alliances with other hospital departments, pioneered studies of critical community issues, and developed and published models for social health principles that have achieved national and international acclaim. The department pioneered a hospital patient representative program, introduced a social health advocacy program, and developed and maintains an active liaison between the medical center and a range of social support services in the community.

Foundation of the Medical School and the Department of Community Medicine. The Mount Sinai School of Medicine and its Department of Community Medicine can also be viewed as products of their times. The medical school was founded, in 1966, at a time "when medical education was in the uneasy position of preparing physicians to reconcile the conflicting demands of a society that articulated a strong faith in the power of medical science to conquer disease, but at the same time expressed loss of confidence in the capacity of physicians to control health and medical affairs with equity, efficiency, and compassion" (6). The philosophy of the School of Medicine is derived from the hospital's tradition and practice of social commitment.

The Department of Community Medicine, with its interdisciplinary and multiprofessional staff, was established in the School of Medicine in 1968, lodging the community components of education, service, and research in a "third faculty" representing the community.

Outreach and Partnership through the Department of Community Medicine. From 1968 through to the present, the department has interfaced with local communities, with environmental and occupational communities, and with public or community health programs all over the world. Under the same leadership and vision for the vast majority of its 24-year history, the department has had a clearly articulated policy and philosophy of helping people help themselves and is "unique among community medicine programs in the United States in having enlisted the continuing support of other medical center departments and in establishing collaborative relationships with the East Harlem community [to accomplish this goal]" (7).

The Department of Community Medicine participates with a broad variety of East Harlem community groups representing both consumer and provider in a continuing process of identifying and solving health and health-related problems. In partnership with these groups, the department "encourages a more rational utilization of existing resources in the area, fosters interorganizational linkages, mergers and agreements, and has as an ultimate goal the regionalization of health services in the area" (8). Social workers and social-health principles are woven into the fabric of the community projects and programs conducted or supported through the department.

The Department of Community Relations: "New Kid on the Block." By Mount Sinai standard of longevity, in which major milestone anniversaries of departmental existence (such as the Department of Social Work Services' 85th anniversary in 1991) and staff employment tenures of 25, 35, and 40 years are commonplace, the Department of Community Relations, established in 1985, is relatively new. It, too, is a product of its time, born of the intent to increase medical center liaison with community-based health, social service, and civic organizations and schools and political leaders in East Harlem and nearby communities. Directed by a social worker but with line responsibility in an administrative division of the medical center, this department reflects Mount Sinai's commitment to strong liaison and linkage with the community. Since inception of the department, Mount Sinai has increased its already substantial participation in and partnership with a cross-section of East Harlem community committees, boards, and coalitions as well as coalitions and groups in the broader community.

Other Departments' Commitment to Community Partnership. Many other Mount Sinai departments are and have been actively involved in community outreach and in developing partnerships with individuals and organizations in the East Harlem community, including the departments of Pediatrics, Nursing, Neurology, and Psychiatry. Levels of involvement range from intensive integration in all facets of a particular segment of the population's needs—such as Pediatrics' involvement with child and adolescent treatment, prevention, and education programs—to single, grant-funded projects that also find the community a rich source of research data, which may lead to long-term funding of critical programs.

The Department of Health Education, with funding from an endowment by the Edith K. Ehrman East Harlem Health Education Center, established in 1987, provides technical assistance to East Harlem community health providers in the design and implementation of health education programs, a productive partnership in the community.

Model of Social-Health Integration. The vision of hospital, medical, and social work leaders at Mount Sinai helped to create and sustain a model of social-health integration in hospital and community programs that is unique in the United States. In 1967 Mount Sinai broadened its Medical Board representation to include social work, in recognition of the psychosocial components inherent in all medical care. Social work was included in Mount Sinai's new medical school, and its placement in the Department of Community Medicine was seen as an essential component of that department's ability to address the problems of diverse populations in a given community. "In spirit and in fact, it [social work as a component of the medical school] represents the first tangible expression of an institutional commitment to understand and respond to community needs. The Department of Community Medicine is an interprofessional articulation of the same commitment" (9).

Consumer Participation in and Partnership with the Medical Center. In 1969, the federal government funded an ambulatory care program for medically underserved patients. To be eligible for what became known as "ghetto medicine" funds, hospitals were required to develop mechanisms for community participation in monitoring the program. To that end, Mount Sinai established a community board for ambulatory care, composed of a majority of consumers, and a joint committee on ambulatory care, composed of the Community Board on Ambulatory Care, several members of the Board of Trustees, and staff. The ghetto medicine program was terminated in 1977.

Although the regulatory requirement for consumer participation no longer existed, Mount Sinai recognized the importance of continued consumer input. It charged the Department of Social Work Services with developing a new schema for a board and linkage with the community.

Mount Sinai's Internal Community Board: An Example of Partnership. The Mount Sinai Medical Center Community Board was formulated, and the Board of Trustees approved its bylaws in the fall of 1978.

These bylaws, which continue in effect today, require that at least 51% (14 of 26) of the board be consumer and/or community members, seven of whom are users of Mount Sinai services, six of whom represent social, educational, and welfare (excluding health) agencies in East Harlem and/or are individual East Harlem community residents, and one of whom may be from either one of the two groups. On balance, therefore, this group of consumers represents a broader community than the immediately surrounding East Harlem community.

Of the 12 provider members, five are designated by and representative of administration, the medical school, the Board of Trustees, attending staff, and the Auxiliary Board, and seven are representatives of the Medical Board on Ambulatory Care and the departments of Pediatrics, Medicine, Surgery, Nursing, Social Work, and Community Medicine. The community board is to "serve as a forum to assist and advise the medical center in fulfilling its service, educational, and resources objectives in a manner responsive to consumer health care needs, with emphasis on the needs of East Harlem residents" (10).

According to several members of the community board who were interviewed for this article, the Mount Sinai Community Board has been active within the medical center since its inception. It was particularly instrumental some years back in identifying problems and recommending solutions to problems in delivery of emergency room services. It has reviewed a number of internal proposals and reports from medical center providers during its tenure, providing advice on the impact of proposals on the consumer and noting when its advice has been heeded by the medical center.

The vast majority of members have been on the board since inception. Those interviewed see themselves and the board as having been very activist in the early years, playing a significant role in raising medical center consciousness and sensitivity to consumer concerns, and fostering greater awareness of humanistic values within the medical center. They see the medical center as having been responsive, on balance, to their concerns, and they believe that the board has been able to influence medical center action and, on occasion, policy. Any issues raised by the board were "always investigated, always resolved."

As a group, the board found great purpose and value in its role reviewing certificate-of-need applications for capital expenditures and building projects during the 1980s. Community board members saw this as a significant and vital responsibility. When the state changed its review criteria and increased the dollar limit requiring review, many members believed their sense of purpose and level of contribution to be greatly diminished. They have been searching for another activity focus of similar significance since that time.

Through the years, as a result of continued communication on the board among consumer and provider members and of experience within the medical center, this small band of individuals has become better educated about medical center issues and health care and financial issues, and they have thus become able spokesmen and women about the medical center. They have also taught each other—consumer to provider and provider to consumer—a significant amount about their individual issues and concerns. Each has used the other's data to solve problems internally and externally.

But some members fear they are becoming too comfortable, co-opted perhaps, simply talking to themselves, that in the medical center they are given polite but not serious consideration and are not accorded clout or perceived value. They believe there is opportunity for a more effective partnership between the board and the medical center and between the board and the community, as a conduit from the medical center to the community and back. However, some believe that barriers to such a partnership may exist in the internal and external perception that the board may not, in fact, play a significant role in shaping the mission of the hospital vis-à-vis the community.

The medical center, on the other hand, perceives a tremendous value added by the community board, its activities, and its actions and impact since its inception. The internal community board has been an essential mechanism for ensuring that the medical center stays grounded in its social commitment to the community—an internal watchdog for signals that the medical center is straying from its mission, because its members are, for example, aware of and vocal about consumer perceptions of the medical center, aware of and vocal about signs and symptoms of racial prejudice within the medical center, and vocal about ensuring a one-class system of care within the institution.

These differing perceptions of value are, in part, the consequence of the typical tensions and dilemmas in partnerships. Members of the community board are defining their value in terms of greater responsibility and the desire to be more proactive, with more perceived clout. The medical center perceives value, but recognizes the limitations of power necessitated by the nature of the partnership. It is apparent, therefore, that the board and the medical center must redefine their relationship and bring greater clarity to the definition of this partnership. Those members interviewed believe it essential to:

- reexamine their role
- evaluate their effectiveness on an ongoing basis
- redirect their goals
- become more visible within the medical center
- achieve greater communication with internal departments, the Medical Board, and the Board of Trustees
- redefine internal perception of the Community Board's role as proactive rather than reactive
- develop linkages with internal departments and with community-based organizations
- infuse new blood into the board with greater ties to the community, increasing grass-roots participation where possible
- develop long-term goals for effective board participation in collaborative efforts of the medical center in the community

They also believe it imperative for the medical center to reflect on exactly what continued benefit to the medical center the board has and should have. This is an excellent example of the opportunity the medical center has to eliminate barriers to effective partnerships by, at the minimum, working with the community board to achieve the objectives cited by those recently interviewed and by using these objectives in redefining other partnerships, where appropriate.

Summary and Comment

The Mount Sinai Medical Center, like many academic health centers in urban settings, has traditional roles steeped in clinical, research, and educational imperatives. However, like all voluntary, not-for-profit hospitals, it also has roots in community service, a strong commitment to social-health values, and a long history of community involvement; its staff and departments are directly or indirectly involved in an extensive number of community health programs, projects, committees, and groups. It has a Department of Community Medicine, a Department of Community Relations, an internal Community Board, liaison to and membership on a number of community-based boards and coalitions, and extensive social worker contact with and assignment to community outreach programs. It has the people, the programs, and the tools in place to effectively serve its communities.

In examining the challenges and opportunities that lie ahead, Mount Sinai recognizes that existing capabilities must be enhanced and expanded to bring about more effective partnerships with and collaborative activities within the community. These capabilities need to be effectively connected within the medical center, particular groups or departments serving as the primary link between the community and the medical center as well as within the medical center, particularly for the review and consideration of new or revised programs and services and how such programs and services will affect the community.

The Role of an Enhanced Internal Community Board. An enhanced internal community board, with its consumer and provider membership, could offer greater linkage with and communication between the medical center and the community and between medical center departments and the community. As a primary linkage within the medical center, the community board would be seen by provider and administrative staff members as an opportunity to forge a new community partnership and increase their own participation in shaping the mission of the hospital vis-à-vis the community. Increased communication between and linkage with the Department of Community Relations and the Department of Community Medicine, through representatives on the community board, would be essential. An internal community board could also contribute to evaluation of the effectiveness of medical center community programs and could have more contact with other boards, community groups, and coalitions within the community in order to accomplish such evaluations: Are programs effective? Are resources allocated appropriately? Do opportunities exist for redistribution of resources or realignment of focus? And, finally, it is essential that community board activities and actions be routinely communicated to the Board of Trustees through formal, written minutes and that the community board be perceived in a proactive, rather than reactive, role vis-à-vis the medical center and the community.

In addition, all existing internal networks and networks in all existing community boards, coalitions, and groups must be maximized, and a system of effective, navigable, interconnected linkages developed and clearly communicated to all concerned. Not only must network linkages be elucidated; the relationships inherent in or created by the linkages must be worked, carefully and thoughtfully. The rights and responsibilities of each partner in each of the partnerships must be clearly defined and understood, territorial and control mechanisms must be eliminated, communication efforts must be increased, and communication tools must be targeted effectively.

Commitment to community partnerships must start with the leadership of the institution and the leadership of the community. The renewed values of community commitment must be inculcated in every internal relationship and partnership. The urban medical center must rethink jobs, operating systems, and facilities in terms of its community commitment. It must move from competitive, fragmented planning to formal, integrated planning that is also flexible enough to quickly permit change when change is indicated. It must manage its resources—human, financial, technical, and organizational—proactively and ethically, and it must enlist the community for whom these resources exist to understand the responsibility we all have to use our resources prudently and wisely, targeting the needs of those we serve.

With its professional, technologic, educational, and research capabilities in place, can the urban medical center of the 1990s forge a new era of cooperation and collaboration? Pummeled as we are by complex issues and complicated forces, can we meet the challenge of community partnerships? Can we preserve our resources for a better future despite overwhelming odds?

Yes, if we rediscover our roots in and mission of community service; maximize social-health concepts; eliminate barriers to effective partnerships; "network our networks," by emphasizing and enhancing existing internal and external relationships; maximize existing programs, forums, and organizational structures to create the foundation for new opportunities and innovative partnerships; and ensure that the community's perception of the institution reflects our own articulation of our mission as well as our actions, our service, and our commitment.

Acknowledgments

The authors wish to thank Maryanne Shanahan, RN, BSN, for her participation in the preparation of this article.

References

1. Rehr H. Social work and medicine at Sinai: then and now. In: Rehr H. Milestones in social work and medicine: social health care concepts. New York: Prodist, 1982:51.
2. Davidson D. A decade of competition ends—a new era of cooperation begins. Hospitals 1992; 44–50.
3. Jones SB, DuVal MK. What distinguishes the voluntary hospital in the increasingly commercial health care environment? In: Seay JD, Vladeck BC. In sickness and in health: the mission of voluntary health care institutions. New York: McGraw-Hill, 1988:203.
4. Brenner B. East Harlem health initiatives. Mount Sinai Medical Center: unpublished report. 1991.
5. Rehr H. Social work and medicine at Sinai: then and now. In: Rehr H. Milestones in social work and medicine: social health care concepts. New York: Prodist, 1982:47.
6. Bosch SJ, Deuschle KW. Social work: An important component of community medicine. In: Rosenberg G, Dana B, eds. Social implications in health care in contemporary perspectives. Mt Sinai J Med 1989; 56(6):459–467.
7. Gibson CD, Leaf A, Walker JEC. Mount Sinai Medical Center Department of Community Medicine Academic Review. Report of an external evaluation. New York, 1981.
8. Deuschle KW. Department of Community Medicine, Mount Sinai School of Medicine of the City University of New York, 1968–1980 report. 1981.
9. Dana B. The social-work community medicine connection. In: Rosenberg G, Rehr H. Advancing social work practice in the health care field. New York: Haworth Press, 1983:11.
10. Bylaws of the Mount Sinai Medical Center Community Board, 1978.

Kurt W. Deuschle enjoying goatmeat refreshments following a community meeting, Many Farms, Navajo Nation, 1975.

Hepatitis B Virus Vaccine in The Gambia, West Africa:

Synergy between Public Health Research and Practice

Robert W. Ryder, M.D.

Abstract

Hepatitis b virus infection is endemic in West Africa, and as a result, this region has a high rate of hepatoma. The recent development of an effective, safe vaccine against hepatitis B virus that provides durable immunity may now make feasible the prevention of persistent hepatitis B virus infection and thus the prevention of hepatoma. A comprehensive primary health care program in which over 70% of children in The Gambia, West Africa, receive all their childhood immunizations afforded a unique opportunity to carry out a large public health research program to prevent hepatoma and at the same time strengthen the country's primary health care program.

In 1986, phased introduction of hepatitis B virus vaccine was initiated in The Gambia's Expanded Programme on Immunization. By 1990, 124,577 children had been carefully identified and recruited into the 35-year longitudinal cohort study; 59,803 of these children received hepatitis B virus vaccine. The other 62,774 unvaccinated children will serve as controls. Preliminary studies have demonstrated that the rate of hepatitis B virus infection has fallen to less than 5% in children who received all four doses of hepatitis B virus vaccine.

This study should provide the data from which the protective efficacy of hepatitis B virus vaccine against hepatoma can be accurately determined. In addition, it has energized The Gambia's Expanded Programme on Immunization so that at present more than 90% of all children are receiving all their childhood immunizations. This model cancer prevention program demonstrates the potential synergy between public health research and practice.

The integration of health care into communities that are underprivileged has long been a goal of Kurt Deuschle. Just such a project, an innovative one currently in process in West Africa, involves introducing a vaccine against hepatitis B virus into the primary health care system of The Gambia. The goal of this vaccine trial is to prevent primary liver cancer, one of the most common cancers in the developing world.

Health for All by the Year 2000. Since the 1977 meeting at Alma Alta in which the World Health Organization set the ambitious goal of providing all citizens of the world access to health care by the year 2000, interest in primary health care in less-developed countries has been renewed. Provision of primary health care includes providing access to essential drugs at village-based dispensaries and establishment of maternal and child health clinics, where pregnant women are vaccinated antenatally against tetanus and their children are vaccinated against certain vaccine-preventable infections—tuberculosis, diphtheria, pertussis, tetanus, polio (types 1–3), measles, and yellow fever. This latest

---

From the Department of Community Medicine, Mount Sinai School of Medicine (CUNY), New York, NY. Address reprint requests to the author at Department of Community Medicine, Mount Sinai School of Medicine, Box 1043, One Gustave L. Levy Place, New York, NY 10029.

activity has been carried out by a special program assisted by the World Health Organization, the Expanded Programme on Immunization (EPI). The prevention of these seven early childhood diseases through immunization has been shown to be one of the public health interventions with the highest benefit-to-cost ratio (1).

The Gambia and Its Primary Health Care Program. The hepatitis B virus vaccine trial is taking place in The Gambia, a small country (population 700,000) in West Africa. The Gambia has established one of the most successful primary health care programs in Africa. An EPI program in which over 80% of Gambian children receive all their childhood immunizations has contributed significantly to the success of the primary health care initiative (Table 1). This program was begun in 1979 by the Gambian government. Countrywide vaccination has been carried out by 17 mobile immunization teams who visit 104 delivery points at least once every two weeks. Each newborn child receives an "Infant Welfare Card" on which the dates of all subsequent vaccines are recorded.

Using routing cluster sampling to assess rates of vaccination, The Gambian EPI showed that in 1986 93% of eligible infants had received their first DPT vaccination and about 60% had been fully vaccinated (2).

Hepatitis B Virus Infection and Primary Liver Cancer in The Gambia, West Africa. In the mid-1980s the development of safe, effective subunit vaccines against hepatitis B virus led to suggestions that this vaccine should be incorporated into the EPI program (3). In West Africa, including The Gambia, nearly everyone is infected with hepatitis B virus during childhood (4). In contrast to the situation in Japan and Taiwan, where a high proportion of infants are infected perinatally, infection of newborns is uncommon in West Africa (5–8). During the first two years of life, however, infection rates increase rapidly; by the age of 2 years, 40% of the children have already been infected with hepatitis B virus and 15% have developed persistent infection. By the age of 10 years, 90% of the children have been infected and 20% have become chronic carriers. Acute disease caused by hepatitis B infection has not been recognized as a major problem in The Gambia. The high prevalence of the HbsAg carrier state is believed, however, to be the major risk factor for chronic liver disease and liver cancer.

Sub-Saharan Africa, including The Gambia, has one of the highest rates of hepatoma in the world. In Mozambique, the annual age-adjusted incidence rate for hepatoma is 99.3/100,000 in men and 31.7/100,000 in women (by contrast, the rate in England and Wales is 0.9/100,000 for men and 0.8/100,000 for women) (9). In The Gambia, the annual risk for men and women over 39 years of age is 1.4% in men and 0.3% in women (4). In Africa, individuals in this age group play an important role in the financial and social well-being of the often large families in this region. Prevention of hepatoma could provide substantial benefits to the extended African family.

Beginning with the landmark studies of Beasley in Taiwan in which individuals with persistent hepatitis B virus infection were shown to have a relative risk of developing hepatoma of 273 compared to age-matched, persistently negative individuals, strong evidence has emerged for an etiologic association between persistent hepatitis B virus infection and hepatoma (10). This information is based on retrospective and prospective seroepidemiologic studies, on demonstration of hepatitis B virus DNA integration into host-cell DNA in liver tumors, and on several animal models in which viruses closely related to hepatitis B virus can reproducibly induce hepatoma (10–14). It is thought that the disorganization and rearrangement of hepatocellular DNA that occurs in the setting of hepatocyte necrosis followed by inflammation and subsequent cell regeneration increases the risk of hepatoma, since the hepatocyte becomes more susceptible to genetic alteration (15).

First Large-Scale Trial of a "Cancer-Vaccine": The Gambian Hepatitis Intervention Study

Because of the high rates of early childhood hepatitis B virus infection in The Gambia, the concurrently high rate of hepatoma, and the country's highly successful integration of early-childhood vaccines into the primary health care program, a plan was formulated to integrate hepatitis B virus into this immunization program (16). The aim of this program, called The Gambia Hepatitis Intervention Study (GHIS), was to evaluate whether the prevention of persistent infection with hepatitis B virus by immunization would prevent chronic liver disease, particularly hepatoma. While the original goals of the EPI had been to decrease early-childhood mortality from vaccine-preventable diseases, integration of hepatitis B virus vaccine into the EPI offered the possibility of extending these benefits to adults, particularly men. Decreasing adult male mortality would improve the financial and social well-being of African families, who are often adversely affected by this highly prevalent (particularly in men) and fatal disease.

Immunogenicity of Hepatitis B Virus Vaccine. Before integration could be achieved, extensive planning and additional information on the immunogenicity of hepatitis B virus in Gambian children was required. Previous studies had shown that to be properly protected, children had to receive at least four doses of hepatitis B virus vaccine in the first year of life. The vaccine had to be refrigerated, but not frozen, prior to use. The vaccine was also extremely costly. It has been estimated that immunization of newborns in the high-risk areas of Africa and Asia would cost in excess of $8500 million a year for vaccine alone (17). This figure compares with current World Health Organization (WHO) spending on all immunization programs, about $85 million a year. Furthermore, reliable data on the efficacy of the subunit vaccines in Gambian children was not available. Pilot data were required.

Beginning in 1980, Whittle and his colleagues began a longitudinal study of young children in two isolated villages in a rural section of The Gambia (18). These studies demonstrated high infection rates in the first few years of life. In 1984, Whittle returned to these same villages and, using a variety of doses and routes of injection of hepatitis B virus vaccine, vaccinated all children under 5 years who were seronegative for hepatitis B virus infection. With a regimen of 10 μg of HB_sAg in HBV vaccine given intramuscularly at 1, 2, 4, and 9 months of age, over 95% of the children he vaccinated developed a protective titer of antibodies to the surface antigen of hepatitis B virus. Even though an 89% decay in antibody was demonstrated over the first 2 years, the vaccine was 97% effective in preventing chronic infection in children followed up for at least 4 years (19, 20). This finding was pivotal in the decision to proceed with plans for introducing hepatitis B virus vaccine into the EPI.

Designing the Protocol. However, the manner in which the hepatitis B virus vaccine would be introduced became controversial. Many individuals felt there was no need to carry out a controlled trial of the vaccine's efficacy in preventing persistent hepatitis B virus infection. Because hepatoma is rare in West Africa in men and women under the age of 30 years, a controlled trial of HBV vaccine would require an unacceptably long time. These individuals argued that rather than a controlled trial, a mass demonstration project should be carried out in which hepatitis B virus vaccine would be introduced into the entire EPI program at one time. However, a major obstacle to such a campaign, not only in the Gambia but also in other sub-Saharan countries, was the high cost of the vaccine. A meeting at WHO on the prevention of liver cancer summarized this ethical dilemma well:

> it would be undesirable to consider immunization on [a] wide scale without, at the same time, organizing studies that were designed to establish whether or not the vaccine was producing a reduction in the incidence of the carrier state and subsequently cancer. The best method of ensuring that a protective effect of immunization would be measured would be to conduct a randomized controlled trial. Such a randomized trial would present ethical problems if sufficient vaccine were available to vaccinate all newborn children. At present and probably for the next several years, however, this is not the position as the vaccine is expensive and supplies are limited. (3)

A plan was devised that resolved this dilemma. At least four important considerations dictated the final program (16). First, only limited amounts of vaccine were available, which precluded immediate universal vaccination. Second, it would be desirable to demonstrate the vaccine's efficacy by having both a vaccinated and nonvaccinated control group. Third, given the logistic difficulties of delivering vaccines at each of the 104 delivery points, and with four vaccine doses per child being required, randomization at the level of the individual would be logistically impossible. Randomization would occur at the level of the health zone. Finally, Gambian government officials felt strongly that at the end of any study on vaccine efficacy, hepatitis B virus vaccine would have to be delivered nationally.

With these restrictions and considerations, it was decided to introduce the vaccine in a phased manner, vaccination team by vaccination team at approximately 10- to 12-week intervals so that after four years, national coverage would be in effect. The evaluation of the protective effect of hepatitis B virus vaccination against liver cancer would be made among those who were given hepatitis B virus vaccine and those who were not (16).

For the trial to work, a reliable system was needed to distinguish children who received hepatitis B virus vaccine from those who did not (16). This system would still have to be operative 30 or 40 years later, when the vaccine cohorts reached the age of peak hepatoma incidence. This problem of identification was further complicated by the fact that in The Gambia individuals sometimes change their names and residences. An identification system was finally settled upon that used the name, sex, birth date, and village of residence of each child enrolled into the cohort. A modification was also made in the body site of BCG vaccination, which leads to an easily visible scar in most African children. Children given the combined vaccine received it at one body site; children not receiving the hepatitis B virus vaccine received their BCG at another body site. Finally, a handprint and a footprint would be obtained from each child at the age of four months. Like fingerprints, the patterns of hands and feet, set down in utero, do not change, except in size, throughout life.

Based on the 1981 census carried out in The Gambia, it was estimated that during the four-year period of stepwise introduction of hepatitis B virus, 120,000 children would be born. Therefore, 60,000 children would receive hepatitis B virus vaccine and 60,000 children would not. After adjusting for a number of assumptions, including the percentage of eligible children who would receive one, two, three, or four vaccines, the protective efficacy of each dose of vaccination, the number of children who would be persistently infected during the perinatal period, the proportion of all cases of hepatoma occurring in subjects under the age of 50 years, and attrition rates, it was calculated that slightly over 35 years would be required to obtain unequivocal results (16).

Initiation of Vaccination. Hepatitis B virus vaccine began to be integrated into the EPI program in 1986 (Table 2). At that time 25 teams were delivering vaccine. This number increased to 41 in the following year.

When eligible children came to an immunization clinic for the first time, an Infant Welfare Card was given to each child's mother. The card had been adapted so that a tear-off sheet was created with spaces provided for each child's name, date of birth, mother's name, father's name, village of birth, clinic attended, date of attendance, and registration number. This information was returned to a central office where it was computerized, creating a complete national immunization register.

Early Findings. Between 1986 and 1990, 124,577 children were recruited into the study, of whom 59,803 received hepatitis B virus vaccine. During this period, selected groups of vaccinated and unvaccinated children were tested for hepatitis B virus markers (21). At one year of age, 764 of the 1,041 children who had received hepatitis B virus vaccine were traced, and blood was taken. Of these 764 children, 710 (93%) were found to be anti-HBsAG positive/anti-HBcAg negative. Since hepatitis B virus vaccine has been engineered to provide antibodies against surface antigen (previously shown to provide protection against infection) and since antibody to core antigen is of long duration and induced only by natural infection, the anti-HB_sAg positive/anti-HB_cAg negative profile in vaccinated children was considered evidence of an effective, protective response to the vaccine. Only 2 of the 710 children had potential persistent hepatitis B virus infection, and 15 (2%) had no detectable anti-HB_sAg (that is, were nonresponders to the vaccine). These data indicate that, at least in the first year following vaccination, hepatitis B virus vaccine was extremely effective.

The high protective efficacy of hepatitis B virus vaccine in Gambian children is shown in a study of 526 uninfected, unvaccinated children aged 30 months to seven years conducted concurrently with introduction of the hepatitis B virus vaccine. In this study, 160 (30%) of the 526 children became infected with hepatitis B virus during a two-year follow-up period (22).

Hepatitis B virus vaccine coverage surveys have been conducted continuously since the initiation of hepatitis B virus vaccination. These surveys have consistently demonstrated that 98% of children aged 12–18 months had received the first dose of vaccine, 94% the second, 92% the third, and 74% the fourth.

After four years, the GHIS program has demonstrated that hepatitis B virus vaccine can be successfully integrated into the EPI program (21). Preliminary data also suggest that the vaccine will dramatically decrease the prevalence of persistent hepatitis B virus infection in fully vaccinated subjects.

At present, a nationwide cancer registry is being set up in anticipation of the first cases of hepatoma that will occur in the study cohort as early as the age of 10 years. When cases of liver cancer or liver disease are detected in someone of the appropriate age, extensive efforts will be made to determine whether the subject is included in the study. Use of the nationwide immunization data bank should then enable those subjects who received a full course of hepatitis B virus vaccine and those who did not receive hepatitis B virus vaccine to be identified. These data should permit an analysis of the protective effect of a full course of hepatitis B virus against hepatoma (21).

Summary

The GHIS is an ambitious undertaking. It consists of a 30- to 40-year controlled trial of hep-

TABLE 1
Immunization Schedule Used in the Expanded Programme on Immunization in The Gambia, 1986

Age	Vaccine
Birth or soon after	BCG; polio 1
2 mo or later	DPT 1; polio 2
1 mo later (≥3 mo)	DPT 2; polio 3
1 mo later (≥4 mo)	DPT 3; polio 4
9 mo or later	Measles; polio 5; yellow fever

BCG, bacille Calmette-Guérin; DPT, diphtheria-pertussis-tetanus.

TABLE 2
Immunization Schedule for the Expanded Programme on Immunization (EPT) following Introduction of Vaccine against Hepatitis B Virus

Age (months)	Routine EPI vaccines	HBV vaccine injection
Birth*	BCG, polio 1	1
2	DPT, polio 2	2
3	DPT, polio 3	
4	DPT, polio 4	3
9	Measles, yellow fever, polio 5	4

* Within 1 month of birth.

atitis B virus vaccine to determine whether this vaccine will prevent hepatoma, a common cause of death in adult Africans. The design of the study should provide the data from which the protective efficacy of hepatitis B virus vaccine against hepatoma can be accurately determined (21). However, the vaccination program has done and will continue to do more than provide vaccination. It has provided a real boost to The Gambia's already successful EPI program. Although vaccine coverage rates were approximately 70% before implementation of the GHIS program, they are now over 90%. In addition, the use of a vaccine to prevent a common cancer has energized The Gambia's entire primary health care program. Gambian health workers from the village level up through the head of the nation's public health program have shown renewed desire to provide primary health care for all Gambians.

There is every reason to believe that the GHIS trial will convincingly demonstrate that hepatitis B virus vaccine will prevent hepatoma, but a negative result will not mean that the program was a failure. Rather, this program will have moved The Gambia one step closer to the goals of Alma Alta, "health for all by the year 2000." The combination of sound public health research at the primary health care level with the actual boost to the practice of public health that the GHIS program has provided serves as an example of the type of work that has characterized Dr. Deuschle's professional life.

Acknowledgments

Much of the material in this manuscript was originally compiled by members of the Gambian Hepatitis Study Group, and I acknowledge the work of this group not only for their succinct reports on the trial, but also for their efforts in the implementation and conduct of The Gambian Hepatitis Intervention Study.

References

1. Foster SO. Immunizable and respiratory diseases and child mortality. Population Dev Rev 1984; 10(Suppl): 119–140.
2. The Republic of The Gambia. Annual EPI coverage evaluation, 1986.
3. World Health Organization. Prevention of liver cancer. Technical Report Series 691. Geneva: WHO, 1983.
4. Ryder RW, Whittle HC, Sanneh ABK, et al. Persistent hepatitis B virus infection and hepatoma in The Gambia, West Africa: a case-control study of 140 adults and their 603 family contacts. Am J Epidemiol (in press).
5. Barin F, Perrin J, Chotard J, et al. Cross-sectional and longitudinal epidemiology of hepatitis B in Senegal. Prog Med Virol 1981; 27:148–162.
6. Okada K, Kamiyami I, Inomata M, et al. e entigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. N Engl J Med 1976; 294: 746–749.
7. Foster O, Ajdukiewicz A, Ryder RW, et al. Hepatitis B virus transmission in West Africa: a role for tropical ulcer. Lancet 1984; 2:449–452.
8. Marinier E, Barrois V, Larouze B, et al. Lack of perinatal transmission of hepatitis B virus infection in Senegal, West Africa. Trop Paediar 1985; 106:843–848.
9. Waterhouse J, Muir C, Shanmugaratnam K, Powell J, eds. Cancer incidence in five continents, Vol 4. IARC Scientific Publications No. 42. Lyon, France: International Agency for Research on Cancer, 1982.
10. Beasley RP, Hwang L-Y, Lin C-C, et al. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22,707 men in Taiwan. Lancet 1981; 2:1129–1133.
11. Trichopoulos D, Tabor E, Gerety RJ, et al. Hepatitis B and primary hepatocellular carcinoma in a European population. Lancet 1978; 2:1217–1219.
12. Tabor E, Gerety RJ, Vogel CL, et al. Hepatitis B virus infection and primary hepatocellular carcinoma. J Natl Cancer Inst 1977; 58:1197–1200.
13. Shafritz DA, Shouval D, Sherman HI, et al. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. N Engl J Med 1981; 305:1067–1073.
14. Summer J, Smolec JM, Snyder RA. Virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc Natl Acad Sci USA 1978; 75:4533–4537.
15. Wands JR, Blum HE. Primary hepatocellular carcinoma. N Engl J Med 1991; 325:729–731.
16. Gambia Hepatitis Study Group. The Gambian hepatitis intervention study. Cancer Res 1987; 47:5782–5787.

17. Arthur MJP, Hall AJ, Wright R. Hepatitis B, hepatocellular carcinoma and strategies for prevention. Lancet 1984; 1:607–610.
18. Whittle HC, Bradley AK, McLoughlan K, et al. Hepatitis B virus infection in two Gambian villages. Lancet 1983; 2:1203–1206.
19. Whittle HC, Inskip H, Hall AJ, et al. Vaccination against hepatitis B virus and protection against chronic viral carriage in The Gambia. Lancet 1991; 337:747–750.
20. Whittle HC, Inskip H, Hall AJ, et al. Vaccination against hepatitis B and protection against chronic viral carriage in The Gambia. Lancet 1991; 337:747–750.
21. The Gambia Hepatitis Study Group. Hepatitis B vaccine in the expanded programme of immunization: the Gambian experience. Lancet 1989; 1:1057–1059.
22. International Agency for Research on Cancer. The Gambia Hepatitis Intervention Study (GHIS). Annual Report: 1991. Lyons, France.

The Epidemiology of HIV Infection and AIDS in East and Central Harlem, NY

David N. Rose, M.D.

Abstract

This report describes the AIDS epidemic in East and Central Harlem, among the hardest-hit communities in the nation. Information was obtained from two New York State mandatory reporting programs: anonymous HIV antibody testing of newborns, and physician and hospital reports of AIDS cases to city and county public health departments. One of 30 babies born in East Harlem and one of 46 newborns in Central Harlem are seropositive. The cumulative rate of reported AIDS cases in these communities is 10–15 times the national rate, and together, the communities reported 1.3% of all AIDS cases in the nation, although they have only 0.1% of the nation's population; 2.2% of all childhood AIDS cases have been reported from East and Central Harlem. Women, minorities, and injection drug users comprise a higher proportion of the cases than in the city, the state, and the nation. The consequences of the epidemic in these communities are enormous, including profound stresses on community institutions and exacerbation of the resurgence of tuberculosis.

The acquired immunodeficiency syndrome (AIDS) epidemic is now beginning its second decade. Perhaps the most notable manifestation of the epidemic today in this country is its disproportionate growth among minorities, injection drug users, women, and children. This report describes the AIDS epidemic in two adjacent neighborhoods that are among the hardest hit in the nation, where these manifestations are most noticeable.

Since AIDS was first described in 1981 (1), the epidemic has raged in East and Central Harlem worse than in most other communities of the United States. Indeed, 6.5% of all AIDS cases in New York State and 1.3% of all AIDS cases reported in the United States come from these two neighborhoods alone. The consequences of the epidemic on the life of these communities and the demands on health care resources and other community institutions are staggering.

Methods

Information on the epidemic in these communities was obtained from two New York State mandatory reporting programs: anonymous HIV antibody testing of blood specimens to detect newborn hereditary disorders, and physician and hospital reports of AIDS cases to city and county public health departments. Newborn seroprevalence data were obtained from the New York State Department of Health Newborn Seroprevalence Study. The method of newborn HIV antibody testing has been reported elsewhere (2). When the zip code of the mother's residence was unknown, the hospital's zip code was substituted. AIDS data were obtained from the New York City Department of Health AIDS Surveillance Unit, the New York State AIDS Epidemiology Program, and the Centers for Disease Control, Division of HIV/AIDS. Vital statistics were obtained from the New York City Department of Health Bureau of Vital Statistics and other sources (3–7).

East Harlem is defined here as zip codes

From the Departments of Community Medicine and Medicine, Mount Sinai School of Medicine (CUNY), and the AIDS Center, Mount Sinai Hospital, New York, New York.

Address reprint requests and correspondence to David N. Rose, M.D., Box 1009, Mount Sinai Medical Center, New York, NY 10029.

10029, 10035, and 10037, which includes Wards and Randall's Islands. Central Harlem is defined as zip codes 10026, 10027, 10030, 10031, and 10039, which includes Morningside Heights.

Demographics and Vital Statistics of the Communities. East and Central Harlem are poor, residential communities with all the indices of distressed neighborhoods (Table 1). The annual family income is $12,000, in comparison to the $19,000 annual family income for all of New York City. Unemployment is high in these communities, nearly three times the city, state, and national rates. A large proportion of the population has had less than 12 years of education. Half the families with children under five years old in East and Central Harlem have family incomes below the poverty level, and half the population qualifies for Medicaid. The housing stock is poor. In Central Harlem more than 18% of the residential properties are vacant and 60% are owned by New York City because of nonpayment of property taxes. In East Harlem 20% of the residential properties are vacant, 40% are tenements constructed before 1939, and 20% of residential structures are in poor condition.

The health status indices of these communities reflect these social stresses. The proportion of births to adolescents is far higher in East and Central Harlem than in the rest of the city, state, and nation. In 1988, furthermore, the proportion of deliveries following late or no prenatal care was 16.0% in East Harlem and 20.1% in Central Harlem, in contrast to 12.0% in New York City. Deliveries of low-birthweight babies are also more frequent in these communities. As a consequence, the infant mortality rate is very high, twice the national rate.

An important predisposing condition for a community's high prevalence of HIV infection is the prevalence of illicit drug use. It is estimated that 11,050 people in East Harlem and 13,100 people in Central Harlem are injection drug users, or 18.8% and 11.1% of the respective communities' populations. East and Central Harlem residents comprised 11.9% of New York City's substance abuse admissions in 1988. The rate of drug-dependent deaths is three to four times the rate for the city as a whole.

Another conseqence of drug abuse is the prevalence of violent acts. The homicide rate in these communities is two to three times the city's average.

Results

Newborn HIV Seroprevalence. Because maternal IgG antibodies cross the placenta, positive tests for HIV antibodies among newborns reflect

TABLE 1

*Demographics and Vital Statistics of East and Central Harlem**

	East Harlem	Central Harlem	New York City	New York State	United States
Population	98,677	189,538	7,322,564§	17,990,455§	248,709,873§
Proportion black	50%	72%	24%	14%	12%
Proportion Hispanic†	43%	19%	19%	12%	8%
Median income	$11,878‡	$11,595‡	$19,229	$20,540	$17,567#
Unemployment	12.4%	15.4%	5.7%**	5.1%#	5.3%#
<12 years education‖	58.8%	53.9%	39.8%	33.7%	31.4%
Proportion of population on Medicaid	48.6%	62.5%	35.4%	NA	NA
Proportion of births to teenagers	16.1%	16.4%	10.8%	9.4%	12.5%
Low-birthweight births	13.7%	20.8%	9.7%	7.8%	6.9%
Infant mortality rate (per 1,000 live births)	19.5	22.0	13.4	10.8	10.0
Homicide death rate (per 100,000)	41.5	45.9	26.5	12.8	9.0
Drug-dependence deaths rate (per 100,000)	48.6	50.6	16.5	NA	NA

* All statistics from 1988 data, except where noted.
† May also self-classify as black.
‡ 1985.
§ 1990.
‖ 1980.
1989.
** 1987.
NA, not available.

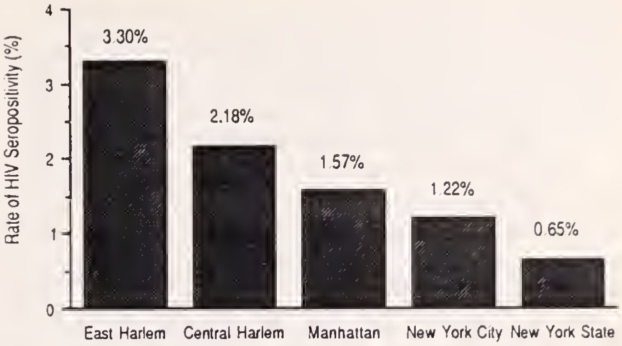
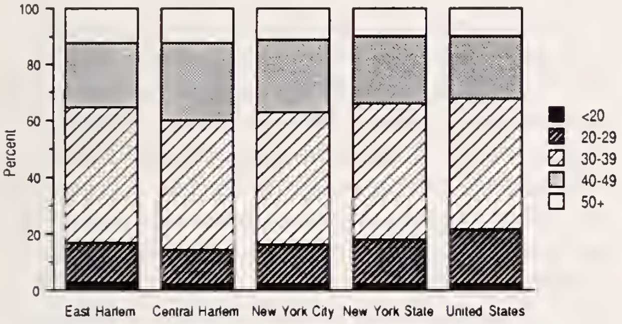


Fig. 1. Prevalence of HIV seropositivity in newborns tested between Nov. 30, 1987, and Dec. 31, 1991.



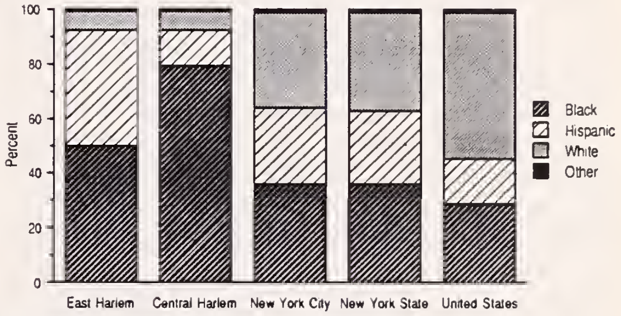


Fig. 2. **Left** Distribution of reported AIDS cases by age group, cumulative through Dec. 31, 1991.
Fig. 3. **Right** Distribution of reported AIDS cases by race/ethnicity, cumulative through Dec. 31, 1991.

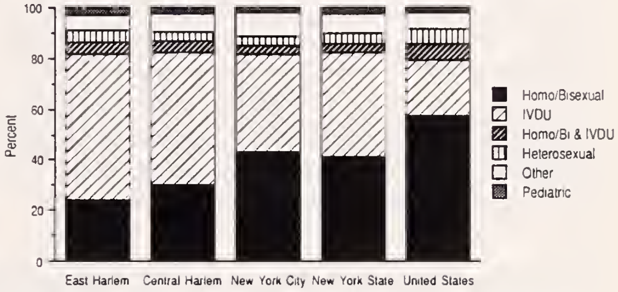


Fig. 4. Distribution of reported AIDS cases by risk group, cumulative through Dec. 31, 1991.

the prevalence of HIV infection in the women of childbearing age in a community. All HIV-seropositive newborns have infected mothers. Approximately 30% of HIV-seropositive newborns have the infection, most of whom manifest illness within their first year of life.

Between Nov. 30, 1987, and Dec. 31, 1991, 334 (3.30%) of 10,123 newborns of mothers with East Harlem residence tested HIV seropositive; 412 (2.18%) of 18,917 newborns of mothers with Central Harlem residence tested HIV seropositive; 1,398 (1.57%) of 89,182 newborns of mothers with Manhattan residence tested HIV seropositive; 6,384 (1.22%) of 522,542 newborns of mothers with New York City residence tested HIV seropositive; and 7,568 (0.65%) of 1,162,268 newborns of mothers with New York State residence tested HIV seropositive (Fig. 1).

Although the race/ethnicity data on newborn seroprevalence are not available for community districts, citywide data are available. Among New York City babies tested between Nov. 30, 1987, and Dec. 31, 1990, 2.22% of black newborns, 1.40% of Hispanic newborns, and 0.32% of white newborns tested positive.

Reported AIDS Cases. East Harlem AIDS cases reported through December 31, 1991, totaled 1,213, including 38 children; 1,563 AIDS cases were reported from Central Harlem, including 40 children (Table 2). These represent cumulative report rates 10–15 times higher than the national rate. Seven percent of New York State's AIDS cases and 1.3% of all AIDS cases in the United States have been reported from these two communities, though they have only 1.6% of the state's population and 0.1% of the nation's population; 2.2% of all childhood AIDS cases have been reported from East and Central Harlem. The proportion of women among persons with AIDS in East and Central Harlem (23.6%) is much higher than city and state proportions and over twice the national proportion. Two-thirds of the reported East and Central Harlem persons have died.

The distribution of AIDS cases by age group and residence (Fig. 2) is as follows: In East Harlem 3.1% of AIDS cases were reported to be in the age group <20 years, 47.7% in the age group 30–39 years, and 12.0% in the age group ≥50 years. The age distribution is similar for Central Harlem: 2.6% in the age group <20 years, 45.0% in the age group 30–39 years, and 12.5% in the age group ≥50 years. The age distributions for the city, state, and nation have slightly smaller proportions of children and older people.

The distribution of AIDS cases by race and residence is illustrated in Fig. 3. In East Harlem half the reported AIDS cases were black, 42.1% Hispanic. In Central Harlem 79.7% of the reported AIDS cases were black and only 12.8% were Hispanic. The racial distribution is more even for the city and state; half the reported cases nationwide were white.

The distribution of AIDS cases by HIV risk group and residence is illustrated in Fig. 4. Injection drug use alone is the predominant risk factor in East Harlem (57.6% of the reported cases) and in Central Harlem (52.0% of the reported cases). By contrast, smaller proportions of the city and state cases are injection drug users and larger proportions are homosexual or bisexual men; homosexual and bisexual men are the predominant group nationwide.

Discussion

East and Central Harlem are extreme examples of the changing nature of the AIDS epidemic: its growth among injection drug users, minorities, women, and children. The dominant mode of spread among men in these communities is injection drug use; among women it is injection drug use and heterosexual contact with injection drug users. As the prevalence rises among women of childbearing age—it is now three to five times higher in these communities than elsewhere in New York State—the prevalence among their children also rises.

New York State has the highest number of AIDS cases reported in the nation, and the majority have been from New York City. Furthermore, Manhattan has had a disproportionate share of the AIDS cases in New York City, with a cumulative incidence 2.5 times higher than the Bronx, the next highest borough (4). In Manhattan, the predominant risk group early in the epidemic was homosexual and bisexual men. In recent years, injection drug use has become the predominant risk behavior, especially in East and Central Harlem. Injection drug use is also the principal transmission factor in women, either directly or through heterosexual contact with injection drug–using men. As a consequence, in New York City, the relative risk of dying of AIDS is 5.1 times higher for black women and 3.7 times higher for Hispanic women than for white women (4). As a further consequence, children born to black and Hispanic women constitute 90% of the childhood AIDS cases in New York City.

Tuberculosis. The AIDS epidemic in these communities cannot be described without consideration of the resurgence of tuberculosis. Reports of tuberculosis cases, rising nationwide since the latter half of the 1980s, have been especially alarming in New York City, with a rate 3.5 times the national rate (8). The AIDS and tuberculosis epidemics have synergistic effects on the community: the people most likely to have dual infection are least likely to have the resources to fight them. HIV-infected people who harbor latent *Mycobacterium tuberculosis* infection are at high risk for developing active disease (9). Those with moderate and severe immunodeficiency manifest

TABLE 2

AIDS Cases Reported through Dec. 31, 1991

	East Harlem	Central Harlem	New York City	New York State	United States
Adults	1,175	1,523	36,574	41,757	202,921
Children	38	40	862	948	3,471
Total no.	1,213	1,563	37,436	42,705	206,392
Rate per 100,000 population	1,229	825	511	237	83
% Female	23.6%	23.6%	15.0%	16.1%	10.5%
% Deceased	67.4%	66.5%	65.2%	66.2%	64.7%
Proportion of AIDS deaths related to narcotics (4)	45.8%	64.4%	32.6%	NA	NA

NA, not available.

active tuberculosis atypically, thereby delaying diagnosis and isolation, putting others at risk for spread. Homeless tuberculosis patients are at especially high risk for interrupting their prolonged course of therapy, thereby putting themselves at risk for the development of resistant *M. tuberculosis* organisms.

Impact. The impact of the AIDS epidemic on East and Central Harlem's life and community institutions has been enormous. Every hospital and community-based health and social service agency in East and Central Harlem has been profoundly affected by the epidemic. In the week of Jan. 19–25, 1992, New York City public and voluntary hospitals reported 2,244 inpatients with diagnosed or suspected AIDS, a 110% increase since March 1987 (10). The epidemic hit at a time when federal funding for neighborhood health centers was declining and state-mandated measures were instituted to decrease the number of hospital beds. Health care institutions have had to deal with a multitude of simultaneous, urgent concerns: the need to develop models of care for a new, chronic illness; the extreme inadequacy of long-term care facilities and substance abuse treatment services; control of occupational HIV and tuberculosis exposures; the shortage of health care workers; and coping with public anxiety about nearly all aspects of the disease.

The human dimension cannot be quantified. Belinda Mason, a member of the National Commission on AIDS, provided some insight before she died of her disease:

> I have to say that people living with AIDS and HIV want nothing more or nothing less than what all of you take for granted today—a place to live, the right to have a job, decent medical care, and to live our lives out without unreasonable barriers. We are not asking for extras, only to be included in what America already delivers to her privileged people. (11)

References

1. Siegal FP, Lopez C, Hammer GS, et al. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative Herpes simplex lesions. N Engl J Med 1981; 305:1439.
2. Novick LF, Berns D, Stricof R, Stevens R, Pass K, Wethers J. HIV seroprevalence in newborns in New York State. JAMA 1989; 261:1745–1750.
3. HIV data book. New York: HSA/NYC, 1991.
4. Milber J, Thomas P, Stoneburner R. Geographic and demographic features of the AIDS epidemic in New York City. NY State J Med 1988; 88:227–232.
5. Murphy D. AIDS/HIV needs assessment of central harlem. New York AIDS Consortium, Oct 18, 1990.
6. National Center for Health Statistics. Vital statistics of the United States, 1988, Vol. I, Natality; Vol. II, Mortality, part A. Washington, D.C.: Public Health Service, 1991.
7. New York State statistical yearbook, 16th ed. Nelson A. Rockefeller Institute of Government, State University of New York, 1991.
8. Mendelson MH, Adler J. Resurgence of tuberculosis: relationship to HIV infection and implications for infection control. Mt Sinai J Med 1990; 57:221–224.
9. Selwyn PA, Hartel D, Lewis VA, et al. A prospective study of the risk of tuberculosis among injection drug users with human immunodeficiency virus infection. N Engl J Med 1989; 320:545–550.
10. Raske KE. Greater New York Hospital Association.
11. National Commission on Acquired Immunodeficiency Syndrome. America living with AIDS. Washington, DC: U.S. Government Printing Office, 1991.

Kurt W. Deuschle presenting medically equipped transport van to Harlem Triangle Association at site of former Mount Sinai buildings on E. 101 St., New York City, December 1970. Beginning second from right: Dr. Hughes, coordinator of the Mount Sinai Department of Community Medicine project; Monsignor Christopher Kane of the Archdiocese of New York, which helped fund the project; Kurt W. Deuschle; Mrs. Kornegay, president of the East Harlem Triangle Association.

Child Labor in the United States:

Historical Background and Current Crisis

Philip J. Landrigan, M.D., M.Sc., Susan H. Pollack, M.D., Renate Belville, M.A., and
James G. Godbold, Ph.D.

For four decades, Kurt W. Deuschle has emphasized in his work and in his writing the importance of assessing disease in its societal context. He has continuously taught us that such study is essential for understanding the etiology of disease as well as for devising robust and rational preventive strategies. We hope that this study of child labor, which had its beginnings in clinical observation and which expanded to include epidemiologic assessment, political commentary, and the formulation of public health policy, represents a fitting extension of Kurt Deuschle's work.

Child Labor

Child labor is defined as the paid employment of children less than 18 years of age. It is a common phenomenon in American society (1). According to provisional data from the U.S. Department of Labor, more than 4 million children in the United States were legally employed in 1988, a substantial increase from a decade earlier (2).

Illegal child labor is also widespread in the United States. Despite the popular belief that this problem was remedied long ago, it has persisted and appears also to be on the rise (2, 3).

Child labor is a major problem internationally (4–7). According to the International Labour Organization, at least 200 million children under the age of 14 are employed worldwide. In some countries, children constitute 15%–25% of the total workforce. Children are employed as rug weavers in the Middle East, as underground tin miners in South America, and as metal workers, fireworks makers, textile weavers, and glass blowers. Injuries, illness, and disability are common among working children worldwide.

Child labor is associated in virtually all countries—both industrialized and developing—with poverty, high unemployment, inadequate educational opportunities, and failure to enforce existing law and standards. Particularly severe abuses have been documented in so-called "free enterprise zones," special industrial areas that have been established in many countries where relaxation has been permitted in the enforcement of labor and environmental laws (4).

Historical Background. Modern-day child labor began in Europe and America during the 1700s as a consequence of the need, created by the Industrial Revolution, for large numbers of workers. In that era, "most mill owners preferred to hire children rather than adults. Above all, they were cheaper . . . but also more tractable, and as labor unions developed, less likely to strike" (8). Children as young as 11, especially girls, were sent by their families to work in the mills because wages they could earn far exceeded the income of their parents at home on rural farms (9).

The hazards and horrors of child labor in the eighteenth and nineteenth centuries were chronicled by Charles Dickens in *Hard Times* (10) and by Frances Trollope in *The Life and Times of Michael Armstrong, the Factory Boy* (11). "Six-year-old girls in the mines [of Scotland] did work that later, in times of relative enlightenment, was turned over to ponies" (12). In the mines of

---

Adapted from Pollack SH, Landrigan PJ, Mallino DL: Child labor in 1990: prevalence and health hazards. Ann Rev Publ Health 1990; 11:359–375.

From the Department of Community Medicine, The Mount Sinai School of Medicine, New York, NY.

Address reprint requests to Dr. Philip J. Landrigan, Dept. of Community Medicine Box 1057, Mt. Sinai Med. Ctr. One Gustave L. Levy Place New York, NY 10029.

Pennsylvania and the mills of Massachusetts and South Carolina, conditions were no different.

> In the spring of 1903, [in] Kensington, Pennsylvania, . . . 75,000 textile workers were on strike. Of this number at least 10,000 were little children. The workers were striking for more pay and shorter hours. Every day little children came into Union Headquarters, some with their hands off, some with the thumb missing, some with their fingers off at the knuckle. They were stooped little things, round shouldered and skinny (13).

Drawings of children being beaten in the cotton mills, lowered on ropes into the coal mines, and carrying 50-pound rocks on their backs up mine ladders sparked great popular revulsion against the worst abuses of child labor, but still these abuses continued (14).

In the United States, despite federal and state legislation, child labor continued to be a major problem during the first third of the twentieth century. Inadequate enforcement of existing statutes contributed to this persistence. The need for enforcement was tragically demonstrated by the Triangle Shirtwaist Fire in New York City in 1911. Late in the afternoon one Saturday in March, a fire broke out on the eighth floor of a building that housed the Triangle Shirtwaist Company. Having recently lost their strike for a 40-hour week, 500 women and children on the seventh, eighth, and ninth floors of the building were still laboring as part of their 59-hour work week. Since "the factory doors were locked each day to keep the workers in and the union organizers out" and the interior doors opened inward, fire escapes seemed the only recourse, but they broke under the weight of the desperate workers. The fire-engine ladders reached only to the sixth floor. Ultimately, 147 young women, many of them under the age of 18, lost their lives that day, only eight years after the passage of landmark child labor legislation and fire protection laws in New York. Many of those killed were the sole providers for their widowed mothers and siblings (15).

In 1938, after several unsuccessful attempts to pass legislation regulating child labor, the Fair Labor Standards Acts was enacted. It remains the major federal legislation governing child labor in the United States today. This legislation established uniform federal standards for minimum wage, overtime pay, and maintenance of records on wages and hours for employees of all ages. Additionally, it established child labor standards, including lists of permitted work hours and prohibited occupations, and it raised the age limit for full-time work to 16.

Under the Fair Labor Standards Act, no child under the age of 16 years may work during school hours, and a ceiling is set on the number of hours of employment permissible for each school day and each school week. Employment in any hazardous nonagricultural occupation is prohibited for anyone less than 18 years old. No child under age 18 may work in mining, logging, brick and tile manufacture, roofing or excavating, or as a helper on a vehicle or on power-driven machinery. Work involving meat-processing machinery, delicatessen slicers, and supermarket box crushers is specifically prohibited. In agriculture, where the restrictions are much less stringent, hazardous work is prohibited only until age 16, and all work on family farms is totally exempted. According to the law, however, no child under the age of 16 working on a nonfamily farm is allowed to drive a tractor with an engine over 20 horsepower or to handle or apply category I or II pesticides and herbicides (16).

Current Extent of Child Labor In the United States

A reconvergence of economic and social factors similar to those that produced the major increases in child labor at the beginning of the Industrial Revolution has produced the current resurgence of child labor in the United States (1, 2). These factors are:

- Increased poverty. Despite a relatively strong economy, more American children live below the poverty line today than 20 years ago. For the 20% of American children who live in conditions of poverty, financial need constitutes a compelling reason to seek employment.
- Unstable world conditions, particularly war and poverty in Central America, the Caribbean, and Southeast Asia, which have led increasing numbers of illegal immigrants to enter the United States. These immigrants, particularly children without parents, are highly vulnerable to exploitation in the workplace because of their overwhelming need for income and their fear of discovery by immigration officials.
- Relaxation since 1980 in enforcement of the federal child labor law, including relaxation of the provisions limiting the maximum permissible hours of work and the prohibitions against use of dangerous machinery. Repeal of the ban on industrial homework that was created 40 years ago to protect working women and children from industrial exploitation in the piecework industry has further undermined the historic intent of child labor law.

The illegal employment of children occurs today in all industrial sectors, and often exists under sweatshop conditions (17, 18). Any establishment that routinely and repeatedly violates laws on wages, hours, and child labor as well as the laws protecting occupational safety and health is termed a sweatshop (19, 20). Traditionally, these shops have been considered fringe establishments, such as those in the garment and meatpacking industries. Increasingly, however, restaurants and grocery stores, not typically considered to be sweatshops, are also fulfilling the definition.

Health and safety conditions in sweatshops are often hazardous. For example, fire hazards may be created by blocked exit doors, accumulations of combustible materials, and inadequate ventilation; electrocution hazards result from overloaded electrical supplies, work stations located close to exposed wire, and bare fuse boxes. The large number of fire code violations being discovered by the inspectors of the Garment Industry Task Force of the New York State Department of Labor suggests that sweatshop workers, including children, are at high risk of dying of fire if these conditions are not alleviated.

Risks of Child Labor

The disadvantages of child labor fall into two categories: (a) threats to education and development and (b) risks of injury, illness, and toxic exposure.

Developmental Risks. One of the principal hazards of child labor is interference with education. Employed children risk having inadequate time for school homework and increased fatigue on school days; teachers of children in areas where preholiday employment is common or industrial work in the home is escalating have reported declines in the academic performances of previously adequate students. These children are described as falling asleep at their desks and unable to learn (21).

Even if they maintain their academic performance, working children are able to participate less actively in after-school activities and sports than their peers. Child labor also interferes with play, which is important for normal development throughout childhood, and relaxation and freedom from fatigue are necessary for children to grow and learn (22, 23).

Health Risk: Injuries. Injuries are the leading cause of death in children older than one year, and they account for 45% of all mortality in 5-to-14-year-old children in the United States. Approximately 10,000 children die from injuries each year (24). Injuries are the leading cause of potential years of life lost (YPLL) in the United States, accounting for 2.3 million YPLL in 1987 (25). The risks of injury and illness associated with child labor appear to pose a significant public health problem, but they have only begun to be explored (26). Almost no data are available to characterize rates of work-related injury among children. Major publications (27, 28) discussing the epidemiology of childhood injury have failed to consider work as a causal or contributing factor. A recent review of adolescent visits to emergency rooms in Massachusetts for treatment of injuries found that of those injuries that had occurred in an identified location, 24% had occurred at work (29).

Delicatessen and bakery slicers, although children under 18 are legally prohibited from using them, have been shown to be sources of serious injury to working children. In the early 1980s a teenage boy in New York City was brought to an emergency room with an amputated arm; he said he had been "helping out" in a butcher shop. A few months later, another teenage boy was brought to the same emergency room with his arm amputated after having been in the same shop. He, too, had just been "helping out" (30). Recently, a 17-year-old girl in New York City amputated several fingers when a bakery dough slicer came down on her hand (31).

The fast-food industry is among the most rapidly growing industries and is one of the largest employers of youth in the United States today. Lacerations and burns are common hazards in fast-food establishments. There is also a risk of electrocution, although this risk may have been lessened by changes mandated subsequent to the death by electrocution in 1987 of a teenage worker in a hamburger restaurant; the source of the boy's electrocution was found to be a power outlet on a wet floor in an improperly grounded building (32).

The delivery of pizzas and other hot food items has proven to be extremely hazardous to working children. The rash promise made by a midwestern pizza company that all pizzas would be delivered within 30 minutes of the time of placing an order has been shown to encourage reckless driving by young, often inexperienced motor vehicle operators. A total of 17 fatalities among children working in pizza delivery and among persons with whom they collided have been documented within the past year to be associated with the ill-conceived delivery policies of this firm (33).

In New York State in the years 1980–1987, Workers' Compensation awards were made to 10,047 children under the age of 18 years for work-related injury; 44% of these injuries resulted in permanent disability. There were 35 deaths (34) (Table 1). These injuries and deaths occurred in a wide variety of industries (Table 2).

Health Risk: Chronic Illness and Toxic Exposure. Little information is available on the incidence or severity of work-related illness in children caused by toxic occupational exposures. Children are, however, known to experience a variety of toxic exposures at work, including formaldehyde and dyes in the garment industry, solvents in paint shops, organophosphate and other pesticides in agricultural work and lawn care, asbestos in building abatement, and benzene in pumping unleaded gasoline. Given the wide occurrence of these exposures, it is not inconceivable that some still undefined fraction of adolescent asthma might be related to occupational exposures to dusts or formaldehyde, or that some cases of leukemia may be the consequence of occupational exposure in adolescence to benzene in unleaded gasoline. Previous studies have shown unequivocally that workers exposed early in life to asbestos can later develop lung cancer and mesothelioma.

Health Risks of Agricultural Child Labor. Rural children are employed extensively in agriculture, both on family farms and commercially. The five hazards to health associated with agricultural work include lacerations, amputations, and crush injuries from farm machinery; blunt trauma from large animals; motor vehicle accidents involving farm vehicles on public roads; risk of suffocation in grain elevators and silos; and exposure to pesticides. Small physical size and inexperience may, however, superimpose an additional risk for young workers.

TABLE 1

Proportion of Permanent Disability by Type of Injury among Workers' Compensation Awards to Children <18 in New York State, 1980–1987

Type of injury	Percent permanent disability*	Total injury awards
Amputations	100%	121
Fractures	68%	1,935
Dislocations	66%	148
Multiple trauma	53%	564
Lacerations	52%	3,340
Gunshot wounds	50%	16
Freezing	50%	6
Other	22%	3,917
	Total	10,047

* Includes 35 deaths from fractures (6), multiple trauma (6), gunshot (3), lacerations (2), poisoning (2), electric shock (1), and unclassified injury (11).

Adapted from data in New York State Workers' Compensation tapes.

TABLE 2

Distribution of Work-Related Injuries in Children by Age and Standard Industrial Classification (SIC) Sector in New York State, 1980–1987

Major SIC sector	Total (%)	Age (%) To 13*	14–15	16–17*
Retail	53	4	17	60
Services	18	3	21	18
Manufacturing	14	91	41	7
Agriculture	5	1	11	4
Wholesale	3	1	2	3
Public administration	2	—	5	2
Construction	2	—	1	2
Transp./comm./electr.	1	†	1	1
Finance/ins./real estate	1	—	1	1
Other	1	†	—	†
Total	100 (10,047)	100 (391)	100 (972)	100 (8,684)

Adapted from data in New York State Workers' Compensation tapes.

* Column total is 100%; some numbers have been rounded.

† ≤.05%.

Although the numbers of children working in agriculture are not so large as in other sectors, the potential hazards (especially those involving machinery and large animals), coupled with the historical lack of regulation of agriculture, combine to create an important problem, particularly in rural states. Agriculture has come to surpass mining as the most dangerous occupation, accounting in 1981 for 61 fatalities per 100,000 workers (35). Perhaps for this reason, much of the scanty literature available on work-related injury and illness in children focuses on agriculture (36–38).

Data on injury in adolescent agricultural workers are provided in a 1985 paper by Rivera (39):

> Nearly 300 children and adolescents die each year from farm injuries, and 23,500 suffer nonfatal trauma. The fatality rate increases with age of the child; the rate for 15- to 19-year-old boys is double that of young children and 26-fold higher than for girls. More than half (52.5%) [of those injured] die without ever reaching a physician; an additional 19.1% die in transit to a hospital, and only 7.4% live long enough to receive inpatient care. The most common cause of fatal and nonfatal injury is farm machinery. Tractors accounted for one half of these machinery-related deaths, followed by farm wagons, combines, and forklifts.

Recommendation for Prevention

Child labor has reemerged in the United States and has become a serious health hazard (40). Prevention of injury and illness in working children requires the following actions:

1. The development of better data to define the extent and severity of the problem of child labor and its associated injuries and illnesses. These data will permit identification of particularly dangerous industries and occupations.
2. Education of children, parents, teachers, physicians, and the business community about the hazards of child labor.
3. Review of existing regulations to determine whether there are areas that require strengthening or modification to be more appropriate to current conditions.
4. Discontinuation of the poorly considered federal initiative to relax certain labor regulations that protect children at work, particularly the regulations limiting industrial homework; this relaxation will lead inevitably to increased child labor.
5. Stricter enforcement of existing law and regulations by state and federal agencies.

Epilogue

On May 29, 1992, a major national conference on child labor was held at the Mount Sinai School of Medicine with the assistance of a special conference grant from the William T. Grant Foundation. The findings and recommendations of this report were presented to a panel of state legislators, staff of the U.S. Congress, and state and federal regulatory officers. We continue to follow the child labor situation closely, and we continue our efforts to foster remedial action at the level of both the individual and the community.

Acknowledgments

The authors acknowledge the generous support of the William T. Grant Foundation and the National Institute for Occupational Safety and Health (Grant #OH-02717).

References

1. Pollack SH, Landrigan PJ, Mallino DL. Child labor in 1990: prevalence and health hazards. Annu Rev Public Health 1990; 11:359–375.
2. Corbin T. Current trends in youth employment. New York State Department of Labor, Division Research Statistics, 1988.
3. Corbin T. Child labor law survey of teenagers. Albany: New York State Department of Labor, Division Research Statistics, Working Paper No. 5, 1988.
4. Albright J, Kunstel M, McKay R. Stolen childhood: a global report on the exploitation of children. Atlanta: Cox Newspaper Enterprises, 1987.
5. Anti-Slavery Society: Child Labour Series. Birmingham, UK: Third World Publ., 1978–1981.
6. Waldron HA. Danger: children at work. Br J Indust Med 1988; 45:73–74.
7. World Health Organization Study Group. Children at work: special health risks. Techn. Rep. Ser. 756. Geneva: WHO, 1987.
8. Trattner WI. Crusade for the children: a history of the National Child Labor Committee and child labor reform in America. Chicago: Quadrangle, 1970.
9. Rosner J. Emmeline. New York: Pocket Books, 1980.
10. Dickens C. Hard times. New York: Norton, 1854/1966.
11. Trollope F. The life and times of Michael Armstrong, the factory boy. London: Colburn, 1840.
12. McPhee J. The crofter and the laird. New York: Farrar, Straus & Giroux, 1970.
13. Zinn H. A people's history of the United States. New York: Harper, 1980:43, 44.
14. Hunter D. The diseases of occupations, 5th ed. London: English University Press, 1974.
15. Wertheimer BM. "We were there"—the story of working women in America. New York: Pantheon.
16. National Child Labor Committee. Child labor and related law compendium. New York, 1986.
17. Bagli CV. Some "hard workers" in garment district are just 12 or 14. New York Observer, Jan 9, 1989.
18. Powell M. Babes in toil-land: child labor and the city's sweatshops. New York Newsday, Jan 8, 1989.
19. U.S. General Accounting Office. "Sweatshops" and child labor violations: a growing problem in the United States. Washington, DC, 1989.
20. U.S. General Accounting Office. Sweatshops in the U.S.—opinions on their extent and possible enforcement actions. Publ. No. GAO/HRD 88-130 BR. Washington, D.C., 1988.
21. New York State Department of Labor. Hearings on Child Labor Law (Albany, Buffalo, Manhattan, Hauppauge and Syracuse), 1988.
22. Cohen S. Social and Personality Development in childhood. New York: Macmillan, 1976:163–186.
23. Campbell SF, ed. Piaget sampler. An introduction to Jean Piaget through his own words. LC75-34129, 1976. pap. 42.00 (ISBN 0-317-0-8167-5, 2020594). Bks Demand UM.
24. Waller AE, Baker SP, Szocka A. Childhood injury deaths: national analysis and geographical variations. Am J Public Health 1989; 79:310–315.
25. Centers for Disease Control. Years of potential life lost before age 65—United States, 1987. MMWR 1989; 38: 27–29.
26. Schober SE, Handke JL, Halperin WE, Mill MB, Thun MJ. Work-related injuries in minors. Am J Industr Med 1988; 14:585–595.
27. Baker SP. Childhood injuries: the community approach to prevention. J Public Health Policy 1981; 2:235–246.
28. Gratz RR. Accidental injury in childhood: a literature review on pediatric trauma. J Trauma 1979; 19:551–555.
29. Anderka M, Gallagher SS, Azzara CA. Adolescent work-related injuries. Presented at Annual Meeting of American Public Health Association, Washington, DC, 1985.
30. Drucker E. Comments on proposed revision of legislation on child labor. Testimony submitted to the Subcommittee on Labor Standards, Committee on Education and Labor, U.S. House of Representatives, 1982.
31. Scott G. Teenager's finger severed in Queens bakery accident. New York Newsday, Sept 29, 1988.

32. National Institute for Occupational Safety and Health, Division of Safety Research, FACE program, Jan 22, 1988.
33. Kelly M. A deadly delivery program. Boston Globe, July 19, 1989.
34. New York State Workers' Compensation Board, 1988.
35. Swanson JA, Sachs MI, Dahlgren KA, Tinguely SJ. Accidental farm injuries in children. Am J Dis Child 1987; 141:1276–1279.
36. Cogbill TH, Busch HM, Stiers GR. Farm accidents in children. Pediatrics 1988; 76:562–566.
37. Broste SK, Hansen DA, Strand RL, Steuland DT. Hearing loss among high school farm students. Am J Public Health 1989; 79:619–622.
38. Karlson T, Noren J. Farm tractor fatalities: the failure of voluntary safety standards. Am J Public Health 1979; 69:146–149.
39. Rivera FP. Fatal and nonfatal farm injuries to children and adolescents in the United States. Pediatrics 1985; 76:567–573.
40. Postol T. Child labor in the United States. Its growth and abolition. Am Educator 1989; 13:30–31.

List of *Journal* Reviewers in 1992

Each year the JOURNAL relies on its reviewers as the linchpin in offering its readers worthwhile, timely, and interesting peer-reviewed articles. The JOURNAL staff thanks the following reviewers for their time and intellectual effort on manuscripts submitted to the publication in 1992.

Edward J. Bottone
Howard W. Bruckner
Lewis Burrows
Nancy E. Davidson
Adrienne M. Fleckman
Etta Friedman
Harriet S. Gilbert
Paul L. Gilbert
Ezra Geenspan
Adrian Greenstein
Richard Haber
Noam Harpaz
Tomas Heimann
Shalom Hirschman
Vincent Hollander
Barry W. Jaffin
Andrew Kaplan
Richard Knight
Kenneth Lieberman
Charles Lockwood
Burt Meyers
Charles K. McSherry
Takao Ohnuma
Jack G. Rabinowitz
Elliot J. Rayfield
Allan Reed
Lewis Reisman
Sheila H. Roman
David N. Rose
Joseph Rovinsky
Allen E. Rubenstein
Henry Sacks
Ira Sanders
Martin H. Savitz
Fenton Schaffner
Michael Serby
Michail Shafir
Alan L. Silver
Allen Silverstein
David M. Simpson
Barry D. Stimmel
Eugene W. Straus
Max W. Sung
Carl Teplitz
Samuel Waxman
Michael F. Wesson
Joseph Wilder
Barnett Zumoff

Subject, Author, and Title Index to Volume 59

Subject, Author, and Title Index to Volume 59

About James F. Holland: 59(5):375–376
Academic legacy of Kurt W. Deuschle, AL Frank, 59(6):447–449
Acidosis: 59(1):1–12
Active specific immunotherapy with vaccine melanoma oncolysate, MK Wallack, SD Scoggin, M Sivanandham, 59(3): 227–233
Acute myocardial infarction in a long-term care institution for the aged, IZ Jimenez-Velazquez, M Rodstein, M Mulvihill, M Hsu, LS Libow, RR Neufeld, 59(1):57–60
Adrenergic receptors in hypertension and heart failure, C Rosendorff, 59(4):281–297
Advances in the treatment for Hodgkin's disease, PH Wiernik, 59(5):405–407
Ahmet T: see Mittelman A
AIDS Grand Rounds: 59(3):244–277
AIDS: 59(1):61–66; 59(3):271–277; 59(3):244–270; 59(6):493–497
Albumin metabolism: a brief review, MA Rothschild, M Oratz, 59(2):155–156
Alcohol and Drug Abuse: 59(2):108–110
Alcoholism: 59(2):108–110
Antenuis A: see Breant B
Antibodies, Monoclonal: 59(3):207–210
Antiprotease: 59(1):43–46
Art of the gastrointestinal consultation: some aphorisms, HD Janowitz, 59(4):341–349
Asfari M: see Breant B
Atesano A: see Breant B
Aufses AH, Kupfer S: David A. Dreiling: in remembrance, 59(3):278–280
Autoantibodies: 59(1):32–37
Awards: Mount Sinai School of Medicine, 1991/92, 59(3):190
Awards: Mount Sinai Journal of Medicine, 1991, 59(3):191

Bacteremia: 59(1):53–57
Barisano A, Mehl B, Bradbury K: Serotonin antagonists, 59(5):432–436
Basic Science: From Bench to Bedside and Back: 59(1):1–12
Basinski A: see Dobosz M
Bauer C: see Sperling RS
Bauman WA, Spungen AM, Raza M, Rothstein J, Zhang RL, Zhong YG, Tsurata M, Shahidi R, Pierson RN, Wang J, Gordon SK: Coronary artery disease: metabolic risk factors and latent disease in individuals with paraplegia, 59(2):163–169
Belville R: see Bloom SW; see Landrigan PJ
Bernard J: Cancer cures yesterday, today, and tomorrow, 59(5):384–386
Bertino JR, Li WW, Lin J, Trippett T, Goker E, Schweitzer B, Banarjec D: Enzymes of the thymidylate cycle as targets of chemotherapeutic agents: mechanisms of resistance: 59(5):391–395
Beta Cells: 59(2):175–185
Bhardwaj S: Monoclonal antibody immunoconjugates in the diagnosis and treatment of advanced melanoma, 59(3): 207–210
Bicarbonate: 59(1):1–12
Blaser MJ: see Shandera WX
Bleiweiss IJ: see Feltinghoff M
Blindness: 59(1):72–74
Bloom SW, Moss J, Belville R, Freidenberg J, Indyk D, Speedling EJ: Community medicine: its contribution to the social science of medicine, 59(6):461–468
Blumenfield S: see Rosenberg G
Boissard C: see Breant B
Bosch S, Fischer EP, Freidenberg J, Merino R: International program in community medicine, 59(6):455–460
Bradbury K: see Barisano A
Brain-gut axis: a model system to study gene regulation, RJ Greenstein, 59(2):135–139
Breant B, Lavergne C, Atesano A, Ferrand N, Asfari M, Boissard C, Antenuis A, Rosselin G: Development of the beta cell, 59(2):175–185
Breast Carcinoma: 59(1):28–31; 59(5):412–415
Brooklyn gastrinomas, EW Straus, JP Raufman, 59(2):125–128
Bruckner HW, Gorbaty M, Lipsztein R, Kranjac G, Lebwohl MG: Treatment of a high-grade neurofibrosarcoma with concomitant vinblastine, doxorubicin, and radiotherapy, 59(5):428–431
Burchenal JH: About James F. Holland: 59(5):375–376
Burger A: see Mandelkern D
Butler GA, Friedman AH: Screening indices for cytomegalovirus retinitis in patients with human immunodeficiency virus, 59(1):61–66

Campylobacter jejuni: 59(1):53–57
Cancer cures yesterday, today, and tomorrow, J Bernard, 59(5):384–386
Carcinoma of the gallbladder: review of the literature and report on 56 cases at the Beth Israel medical center, LH Nadler, CK McSherry, 59(1):47–52
Carcinoma: 59(5):373–436; Breast: 59(1):28–31; Esophageal: 59(1):75–78; Periampullary: 59(1):85–91; Prevention and Treatment: 59(5):387–390
Cardiology Grand Rounds: 59(4):281–325
CB3717: 59(5):419–424
Cell Development: 59(2):170–174; 59(2):175–185
Cerebral Lesions: 59(1):69–71
Cheema-Dhadli S: see Halperin ML
Chemotherapy: 59(5):391–395; 59(5):408–411
Chemotherapy-Induced Emesis: 59(5):432–436
Chemotherapy of metastatic melanoma: traditional and new approaches, SM Cohen, 59(3):220–223
Child labor in the United States: history and current crisis, PJ Landrigan, SH Pollack, R Belville, JG Godbold, 59(6):498–503
Chun H: see Mittelman A
Ciabattoni G: see Patrono C
Clarkson BD: Consistent genetic abnormalities in human cancers as targets for selective therapies, 59(5):400–404
Clinical and genetic associations in Marfan syndrome and related disorders, F Ramirez, B Lee, E Vitale, 59(4):350–356
Clinical Care in Community Medicine: 59(6):439–441
Clinical Management, HIV: 59(3):244–252; Melanoma: 59(3): 203–206

Cobin RH: see Sirota DK
Cody RJ: Newer aspects of neuroendocrine control in heart failure, 59(4):298–308
Cohen SM: Chemotherapy of metastatic melanoma: traditional and new approaches, 59(3):220–223
Cohen CJ: see Segna RA
Colon Obstruction: 59(4):362–365
Community Medicine: 59(6):439–503
Community medicine: its contribution to the social science of medicine, SW Bloom, J Moss, R Belville, J Freidenberg, D Indyk, EJ Speedling, 59(6):461–468
Community partnerships in the 1990s: can urban medical centers meet the challenge? G Rosenberg, H Rehr, S Blumenfield, 59(6):479–486
Congenital Nevus: 59(3):194–197
Consistent genetic abnormalities in human cancers as targets for selective therapies, BD Clarkson, 59(5):400–404
Consultation: 59(4):341–349
Corbalan F: see Gaing AA
Coronary artery disease: metabolic risk factors and latent disease in individuals with paraplegia, WA Bauman, AM Spungen, M Raza, J Rothstein, RL Zhang, YG Zhong, M Tsurata, R Shahidi, RN Pierson, J Wang, SK Gordon, 59(2):163–169
Correlation between lupus anticoagulant and autoantibodies, N Gleicher, L Harlow, A El-Roeiy, 59(1):32–37
Cortical blindness in postpartum preeclampsia progressing to eclampsia: case report, D Mandelkern, A Burger, 59(1): 72–74
Cropley J: see Lehrer S
Cryoblation: 59(4):357–361
Cunningham-Rundles C: Established and new uses of intravenous immunoglobulin, 59(4):335–340
Cytomegaloviruses: 59(1):61–66

Dalton J: see Lehrer S
David A. Dreiling: in remembrance, AH Aufses, S Kupfer, 59(3):278–280; illustration 59(3):280
Davies T: Graves' disease and its retroorbital aspects, 59(4): 326–334
Deligdisch L: see Segna RA
Deuschle, Kurt W.: 59(6):439–503; includes photos
Development of the beta cell, B Breant, C Lavergne, A Atesano, N Ferrand, M Asfari, C Boissard, A Antenuis, G Rosselin, 59(2):175–185
Diabetic Ketoacidosis: 59(1):1–12
Differential diagnosis of pigmented lesions of the skin, M Lebwohl, 59(3):194–197
Diminished ratio of estrogen receptors to progesterone receptors in breast carcinomas of women who have had multiple miscarriages, S Lehrer, E Levine, P Savoretti, J Cropley, J Dalton, HK Song, SN Thung, J Rabinowitz, L Mandell, 59(1):28–31
Dobosz M, Sledzinski Z, Babicki A, Juszkiewicz P, Basinski A, Wajda Z: Synthetic antiproteases in acute pancreatitis: an experimental study, 59(1):43–46
Dottino PR: see Segna RA
Dreiling DA: In remembrance, 59(3):278–280; illustration, 59(3):280
Drug Therapy, Combination: 59(5):428–431
Drug Users: 59(1):69–71
Duodenocolic fistula: an unusual presentation of esophageal squamous cell carcinoma, P Reissman, RM Steinhagen, PF Knight, 59(1):75–78
Dworkin LD: Why kidneys fail, 59(1):13–22
Dysplastic Nevus: 59(3):194–197

Economics of Health Care: 59(6):469–478
Eicosanoids: 59(2):157–162
El-Roiey A: see Gleicher N
Elderly Patients: 59(1):57–60
Emesis: 59(5):432–436
Endometrial Cancer: 59(5):416–418
Endometriosis causing colon obstruction in two menopausal women, JG Petros, N Spirito, R Gosshein, 59(4):362–365
Energy Turnover: 59(1):1–12
Eng J: Exendin peptides, 59(2):147–149
Environmental hazards and public health: lessons for the practice of medicine, RP Wedeen, HE Sheehan, 59(1):23–27
Enzymes of the thymidylate cycle as targets of chemotherapeutic agents: mechanisms of resistance, JR Bertino, WW Li, J Lin, T Trippett, E Goker, B Schweitzer, D Banarjec, 59(5):391–395
Epicardial cryoblation of myocardial tissue using endocardial thermal isolation, ME Lee, 59(4):357–361
Epidemiology of HIV infection and AIDS in east and central Harlem, NY, DN Rose, 59(6):493–497
Ergin A: see Gomes JA
Established and new uses of intravenous immunoglobulin, C Cunningham-Rundles, 59(4):335–340
Estill MR: Ileocecal tuberculosis in an Asian male immigrant: case report and literature review, 59(4):366–370; see Student Prize
Evolution of curative cancer chemotherapy, E Frei III: 59(5): 408–411
Exendin peptides, J Eng, 59(2):147–149

Fahs MC: Public health in crisis: the economic consequences of inaction, 59(6):469–478
Familial adenomatous polyposis: case report and review of the extracolonic manifestations, KA Konsker, 59(1):85–91
Feltinghoff M, Heller DS, Bleiweiss IJ: Ovarian pregnancy with ipsilateral mature cystic teratoma of the ovary: case report, 59(1):82–84
Ferrand N: see Breant B
Festschrift for Rosalyn S. Yalow: hormones, metabolism and society, EW Straus, editor, 58(2):95–185
Fischer EP: see Bosch S
Fistula, Intestinal: 59(1):75–78
Folate Metabolism: 59(2):150–154
Formulary: Serotonin antagonists: treatment of chemotherapy-induced emesis, A Barisano, B Mehl, K Bradbury, 59(5):432–436
Frank AL: Academic legacy of Kurt W. Deuschle, 59(6):447–449
Freidenberg J: see Bosch S; see Bloom SW
Frei E III: Evolution of curative cancer chemotherapy, 59(5): 408–411
Freireich EJ: Impact of cytogenetics and molecular genetics on leukemia therapy, 59(5):396–399
Friedman AH: see Butler GA
From Bench to Bedside and Back: 59(1):1–12
Futterweit W: see Sirota DK

Gaing AA, Corbalan F, Weinberger J: Phycomycosis (mucormycosis) in differential diagnosis of cerebral mass lesions in intravenous drug users, 59(1):69–71
Gallbladder Carcinoma: 59(1):47–52
Gastrinomas: 59(2):125–128; 59(5):425–427
Gastroenterology: 59(4):341–349
Gastrointestinal Regulation: 59(2):117–124; 59(2):125–128; 59(2):129–134; 59(2):135–139
Gene Regulation: 59(2):135–139
Genetics: 59(5):396–404
Genetic Screening: 59(2):108–110
Genetics, Marfan Syndrome: 59(4):350–356
Girma K: see Nilsson G
Gleicher N, Harlow L, El-Roeiy A: Correlation between lupus anticoagulant and autoantibodies, 59(1):32–37
Glick SM: Research in a hierarchy of values, 59(2):102–107
Globus Prize, 1991: ZM Marom, Role of mast cells in bronchial asthma: mechanisms and possible therapeutic indications, 59(3):191
Godbold JG: see Landrigan PJ

Gomes JA, Winters SL, Ergin A: Management of malignant ventricular tachyarrhythmias, 59(4):318–325
Gorbaty M: see Bruckner HW
Gordis E: Science and social policy, 59(2):108–110
Gordon SK: see Bauman WA
Gosshein R: see Petros JG
Grand Rounds: Primary care for patients with HIV, JR Masci, 59(3):253–262; Immunoregulation, immune defects, and clinical strategies in HIV infection, HC Lane, 59(3):244–252; Pulmonary complications of HIV infection, MJ Rosen, 59(3):263–270; HIV infection: the dominant new face of tuberculosis, LB Reichman, 59(3):271–277; Why kidneys fail, LD Dworkin, 59(1):13–22; Graves' disease and its retroorbital aspects, T Davies, 59(4):326–334; Established and new uses of intravenous immunoglobulin, C Cunningham-Rundles, 59(4):335–340; Pathophysiology and treatment of hypertensive heart disease, RA Phillips, 59(4): 309–317; Newer aspects of neuroendocrine control in heart failure, RJ Cody, 59(4):298–308; Management of malignant ventricular tachyarrhythmias, JA Gomes, SL Winters, A Ergin, 59(4):318–325; Adrenergic receptors in hypertension and heart failure, C Rosendorff, 59(4):281–297
Graves' disease and its retroorbital aspects, T Davies, 59(4): 326–334
Greenspan EM: Past—prelude to the future, 59(5):412–413
Greenstein RJ: Brain-gut axis: a model system to study gene regulation, 59(2):135–139
Growth Hormone: 59(2):175–185

Halperin ML, Kamel KS, Cheema-Dhadli S: Lactic acidosis, ketoacidosis, and energy turnover: "figure" you made the correct diagnosis only when you have "counted" on it, 59(1):1–12
Halton KP: Radiological considerations in diagnosis of metastatic melanoma, 59(3):211–216
Harlow L: see Gleicher N
Harrison MJ: see Ralph Colp Prize
Hassett J: see Sperling RS
Heart Failure: 59(4):281–308
Heller DS: see Feltinghoff M
Hemodynamics: 59(1):43–46
Hepatitis B virus vaccine in The Gambia, West Africa: synergy between public health research and practice, RW Ryder, 59(6):487–492
Histopathologic features of malignant melanoma and other pigmented lesions of the skin, RG Phelps, 59(3):198–202
Histopathology: 59(3):198–202
HIV infection: the dominant new face of tuberculosis, LB Reichman, 59(3):271–277
HIV: 59(1):61–66; 59(3):244–252; 59(3):263–270; 59(3):253–262; 59(3):271–277; 59(6):493–497
HIV-1 seroprevalence in pregnant women testing positive on serologic screening for syphilis, RS Sperling, M Joyner, J Hassett, R Kee, P Wheatley, C Bauer, 59(1):67–68
Hodgkin's Disease: 59(5):405–407
Holland JF: Human cancer: from 'precurable' to curable—In Honor of James F. Holland, MW Sung, T Ohnuma, editors, 59(5):373–415; About—:59(5):375–376; Reminiscences: 59 (5):377–379
Holland JF: Successful successive studies in leukemia and breast cancer, 59(5):414–415
Hsu M: see Jimenez-Velazquez IZ
Human eicosanoid biosynthesis and metabolism, C Patrono, G Ciabattoni, P Patrignani, 59(2):157–162
Human cancer: from 'precurable' to curable: in honor of James F. Holland, MW Sung, T Ohnuma, editors, 59(5):373–415
Humoral antimelanoma immunity: induction by mouse anti-idiotypic mAb, MK-23, and association with survival of patients with advanced melanoma, A Mittelman, C Puccio, H Chun, T Ahmed, 59(3):234–237
Hyperglycemia: 59(1):1–12
Hypertension: 59(4):281–297
Hypertensive Heart Disease: 59(4):309–317
Hyperthyroidism in patients treated with lithium: report of nine cases, DK Sirota, RH Cobin, W Futterweit, RL Segal, DR Jacobs, E Merker, 59(1):79–82
Hypothyroidism: 59(2):111–116

Iberti TJ: see Leibowitz AB
Identification of a novel reductase in folate metabolism, SP Rothenberg, D Knyer, G Solomon, 59(2):150–154
Ileocecal tuberculosis in an Asian male immigrant: case report and literature review, MR Estill, 59(4):366–370
Immunoconjugates: 59(3):207–210
Immunoglobulin, Intravenous: 59(4):335–340
Immunoregulation, immune defects, and clinical strategies in HIV infection, HC Lane, 59(3):244–252
Immunotherapy: 59(3):227–237
Immunotherapy of metastatic melanoma with Interleukin-2, PA Paciucci, 59(3):238–243
Impact of cytogenetics and molecular genetics on leukemia therapy, EJ Freireich, 59(5):396–399
Indyk D: see Bloom SW
Insulin Proliferation: 59(2):175–185
Insulin Resistance: 59(1):1–12
Interface of science and medicine, J Lederberg, 59(5):380–383
International program in community medicine, S Bosch, EP Fischer, J Freidenberg, R Merino, 59(6):455–460
Intravenous fat emulsions and the pancreas: a review, AB Leibowitz, P O'Sullivan, TJ Iberti, 59(1):38–42
Introduction: Kurt W. Deuschle and community medicine: clinical care, statistical compassion, community empowerment, AL Silver, DN Rose, 59(6):439–441
Introductions: Festschrift for Rosalyn S. Yalow: hormones, metabolism, and society, EW Straus, 59(2):95; Human cancer: from 'precurable' to curable: in honor of James F. Holland, T. Ohnuma, 59(5):373–374; Modern management of malignant melanoma, M Shafir, 59(3):192

Jacobs A: Reminiscences of a student of community medicine, 59(6):453–454
Jacobs DR: see Sirota DK
Janowitz HD: Art of the gastrointestinal consultation: some aphorisms, 59(4):341–349
Jimenez-Velazquez IZ, Rodstein M, Mulvihill M, Hsu M, Libow LS, Neufeld RR: Acute myocardial infarction in a long-term care institution for the aged, 59(1):57–60
Journal: see *Mount Sinai Journal of Medicine*
Joyner M: see Sperling RS
Juszkiewicz P: see Dobosz M

Kahn H, Waxman J: Peripancreatic lymph node gastrinoma in a patient with Zollinger-Ellison syndrome, 59(5):425–427
Kamel KS: see Halperin ML
Kee R: see Sperling RS
Kidneys: 59(1):13–22
Knight PF: see Reissman P
Knyer D: see Rothenberg SP
Kochupillai N: Neonatal hypothyroidism in India, 59(2):111–116
Konsker KA: Familial adenomatous polyposis: case report and review of the extracolonic manifestations, 59(1):85–91
Koplan JP, Thacker SB: Kurt W. Deuschle: the clinician in the community, 59(6):444–446
Kranjac G: see Bruckner HW
Kupfer S: see Aufses AH
Kurt W. Deuschle and community medicine: clinical care, statistical compassion, community empowerment, AL Silver, DN Rose, 59(6):439–441
Kurt W. Deuschle as an actor and community medicine as his stage, DE Rogers, 59(6):450–452
Kurt W. Deuschle: the clinician in the community, JP Koplan, SB Thacker, 59(6):444–446

Lactic acidosis, ketoacidosis, and energy turnover: "figure" you made the correct diagnosis only when you have "counted" on it: ML Halperin, KS Kamel, S Cheema-Dhadli, 59(1):1–12
Landrigan PJ, Pollack SH, Belville R, Godbold JG: Child labor in the United States: history and current crisis, 59(6):498–503
Lane HC: Immunoregulation, immune defects, and clinical strategies in HIV infection, 59(3):244–252
Lavergne C: see Breant B
Lebwohl M: Differential diagnosis of pigmented lesions of the skin, 59(3):194–197; also see Bruckner HW
Lederberg J: Interface of science and medicine, 59(5):380–383
Lee B: see Ramirez F
Lee ME, Epicardial cryoblation of myocardial tissue using endocardial thermal isolation, 59(4):357–361
Lefkovits AM: Practitioner's approach to management of an atypical mole, 59(3):203–206
Lehrer S, Levine E, Savoretti P, Cropley J, Dalton J, Song HK, Thung SN, Rabinowitz J, Mandell L: Diminished ratio of estrogen receptors to progesterone receptors in breast carcinomas of women who have had multiple miscarriages, 59(1):28–31
Leibowitz AB, O'Sullivan P, Iberti TJ: Intravenous fat emulsions and the pancreas: a review, 59(1):38–42
Leukemia: 59(5):396–399; 59(5):414–415
Leukemia Cell Lines: 59(5):419–424
Leukotriene: 59(2):157–162
Levine E: see Lehrer S
Libow LS: see Jimenez-Velazquez IZ
Lipsztein R: see Bruckner HW
Lithium: 59(1):79–82
Long-term Care: 59(1):57–60
Luft R: Luft's disease revisited: severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control, 59(2):140–146
Luft's disease revisited: severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control, R Luft, 59(2):140–146
Lupus Anticoagulant: 59(1):32–37

Management of malignant ventricular tachyarrhythmias, JA Gomes, SL Winters, A Ergin, 59(4):318–325
Mandelkern D, Burger A: Cortical blindness in postpartum preeclampsia progressing to eclampsia: case report, 59(1):72–74
Mandell L: Role of radiotherapy in malignant melanoma, 59(3):224–226; also see Lehrer S
Marfan Syndrome: 59(4):350–356
Marom ZM: see Globus Prize
Masci JR: Primary care for patients with HIV, 59(3):253–262
McSherry CK: see Nadler LH
Mehl B: see Barisano A
Melanoma, Malignant: 59(3):192–243
Menopause: 59(4):362–365
Merino R: see Bosch S
Merker E: see Sirota DK
Metabolic Regulation: 59(1):1–12
Metabolism: 59(2):155–169
Metastasis: 59(1):75–78
Methotrexate: 59(5):419–424
Miscarriages and Breast Carcinoma: 59(1):28–31
Mitochondrial Dysfunction: 59(2):140–146
Mittelman A, Puccio C, Chun H, Ahmed T: Humoral antimelanoma immunity: induction by mouse antiidiotypic mAb, MK-23, and association with survival of patients with advanced melanoma, 59(3):234–237
Modern management of malignant melanoma, M Shafir, editor, 59(3):192–243
Monoclonal antibody immunoconjugates in the diagnosis and treatment of advanced melanoma, S Bhardwaj, 59(3):207–210
Moss J: see Bloom SW
Mount Sinai Journal of Medicine: awards, 1992, 59(3):191; list of reviewers, 1992, 59(6):505
Mount Sinai School of Medicine: awards and prizes 1991/92, 59(3):190
Mulvihill M: see Jimenez-Velazquez IZ
Myocardial Infarction: 59(1):57–60
Myocardial Tissue, Cryoblation: 59(4):357–361
Myocardium: 59(2):170–174

N^{10}-propargyl-5,8-dideazafolicacid (CB3717): Inhibitory effects on human leukemia cell lines resistant to methotrexate or trimetrexate, Y Takemura, T Ohnuma, 59(5):419–424
Nadler LH, McSherry CK: Carcinoma of the gallbladder: review of the literature and report on 56 cases at the Beth Israel medical center, 59(1):47–52
Neonatal hypothyroidism in India, N Kochupillai, 59(2):111–116
Neonates: 59(2):175–185
Neufeld RR: see Jimenez-Velazquez IZ
Neuroendocrine Control: 59(4):298–308
Neurofibrosarcoma: 59(5):428–431
Newer aspects of neuroendocrine control in heart failure, RJ Cody, 59(4):298–308
Nilsson G, Romell B, Girma K, Seensalu R: Studies of the proposed mechanism regulating parietal cell emptying, 59(2):129–134
Novel Peptides and Enzymes: 59(2):147–154
Nutritional Iodine Deficiency: 59(2):111–116

Obituary: David A. Dreiling: in remembrance, AH Aufses, S Kupfer, 59(3):278–280
Occupational Medicine: 59(1):23–27
Ohnuma T: Introduction, Human cancer: from 'precurable' to curable: 59(5):373–374; also see Takemura Y
Oratz M: see Rothschild MA
O'Sullivan P: see Leibowitz AB
Outbreak of bacteremic Campylobacter jejuni infection, WX Shandera, MP Tormey, MJ Blaser, 59(1):53–57
Ovarian pregnancy with ipsilateral mature cystic teratoma of the ovary: case report, M Feltinghoff, DS Heller, IJ Bleiweiss, 59(1):82–84

Paciucci PA: Immunotherapy of metastatic melanoma with Interleukin-2, 59(3):238–243
Pancreas: 59(1):38–42
Pancreatitis, Acute: 59(1):43–46
Past—prelude to the future, EM Greenspan, 59(5):412–413
Pathophysiology and treatment of hypertensive heart disease, RA Phillips, 59(4):309–317
Patrignani P: see Patrono C
Patrono C, Ciabattoni G, Patrignani P: Human eicosanoid biosynthesis and metabolism, 59(2):157–162
Peripancreatic lymph node gastrinoma in a patient with Zollinger-Ellison syndrome, H Kahn, J Waxman, 59(5):425–427
Petros JG, Spirito N, Gosshein R: Endometriosis causing colon obstruction in two menopausal women, 59(4):362–365
Phelps RG: Histopathologic features of malignant melanoma and other pigmented lesions of the skin, 59(3):198–202
Phillips RA: Pathophysiology and treatment of hypertensive heart disease, 59(4):309–317
Phycomycosis (mucormycosis) in differential diagnosis of cerebral mass lesions in intravenous drug users, AA Gaing, F Corbalan, J Weinberger, 59(1):69–71
Physiology and pathophysiology of gastrin, JH Walsh, 59(2):117–124
Pierson RN: see Bauman WA
Pollack SH: see Landrigan PJ

Polyposis: 59(1):85–91
Postpartum Eclampsia: 59(1):72–74
Poultry: 59(1):53–57
Practitioner's approach to management of an atypical mole, AM Lefkovits, 59(3):203–206
Pregnancy and HIV-1: 59(1):67–68
Pregnancy, Ovarian: 59(1):82–84
Preventive Medicine: 59(6):439–443
Primary care for patients with HIV, JR Masci, 59(3):253–262
Prostacyclin: 59(2):157–162
Protein kinase C: implications for cancer prevention and treatment, IB Weinstein, 59(5):387–390
Public Health: 59(1):23–27
Public health in crisis: the economic consequences of inaction, MC Fahs, 59(6):469–478
Public Policy: 59(2):102–116
Puccio C: see Mittelman A
Pulmonary complications of HIV infection, MJ Rosen, 59(3): 263–270

Quantitative Analysis: 59(1):1–12

Rabinowitz J: see Lehrer S
Radiological considerations in diagnosis of metastatic melanoma, KP Halton, 59(3):211–216
Radiology: 59(3):211–216
Radiotherapy: 59(3):224–226; 59(5):428–431
Ralph Colp Prize, 1991: MJ Harrison, N Sundaresan, Spinal instrumentation for degenerative diseases of the lumbar spine, 59(3):191
Ramirez F, Lee B, Vitale E: Clinical and genetic associations in Marfan syndrome and related disorders, 59(4): 350–356
Raufman JP: see Straus EW
Raza M: see Bauman WA
Receptors, Adrenergic: 59(4):281–297
Receptors, Estrogen and Progesterone: 59(1):28–31
Reductase: 59(2):150–154
Rehr H: see Rosenberg G
Reichman LB: HIV infection: the dominant new face of tuberculosis, 59(3):271–277
Reissman P, Steinhagen RM, Knight PF: Duodenocolic fistula: an unusual presentation of esophageal squamous cell carcinoma, 59(1):75–78
Reminiscences and Reflections of Rosalyn S. Yallow: 59(2):97–101
Reminiscences of James F. Holland: 59(5):377–379
Reminiscences of a student of community medicine, A Jacobs, 59(6):453–454
Research Agendas: 59(2):108–110
Research in a hierarchy of values, SM Glick, 59(2):102–107
Response of the myocardium to overload stress, SS Schreiber, 59(2):170–174
Retinitis: 59(1):61–66
Ricin-A Chain Immunotoxin: 59(3):207–210
Rodstein M: see Jimenez-Velazquez IZ
Rogers DE: Kurt W. Deuschle as an actor and community medicine as his stage, 59(6):450–452
Role of radiotherapy in malignant melanoma, L Mandell, 59(3):224–226
Romell B: see Nilsson G
Rose DN: Epidemiology of HIV infection and AIDS in east and central Harlem, NY, 59(6):493–497; also see Silver AL
Rosenberg G, Rehr H, Blumenfield S: Community partnerships in the 1990s: can urban medical centers meet the challenge? 59(6):479–486
Rosendorff C: Adrenergic receptors in hypertension and heart failure, 59(4):281–297
Rosen MJ: Pulmonary complications of HIV infection, 59(3): 263–270
Rosselin G: see Breant B
Rothenberg SP, Knyer D, Solomon G: Identification of a novel reductase in folate metabolism, 59(2):150–154
Rothschild MA, Oratz M: Albumin metabolism: a brief review, 59(2):155–156
Rothstein J: see Bauman WA
Ryder RW: Hepatitis B virus vaccine in The Gambia, West Africa: synergy between public health research and practice, 59(6):487–492

Savoretti P: see Lehrer S
Schreiber SS: Response of the myocardium to overload stress, 59(2):170–174
Science and social policy, E Gordis, 59(2):108–110
Science and Medicine: 59(5):380–383
Scoggin SD: see Wallack MK
Screening indices for cytomegalovirus retinitis in patients with human immunodeficiency virus, GA Butler, AH Friedman, 59(1):61–66
Seensalu R: see Nilsson G
Segal RL: see Sirota DK
Segna RA, Dottino PR, Deligdisch L, Cohen CJ: Tamoxifen and endometrial cancer, 59(5):416–418
Serotonin antagonists: treatment of chemotherapy-induced emesis, A Barisano, B Mehl, K Bradbury, 59(5):432–436
Shafir M: Introduction, modern management of malignant melanoma, 59(3):192; Surgical treatment of melanoma: what is controversial and what is not? 59(3):217–219
Shahidi R: see Bauman WA
Shandera WX, Tormey MP, Blaser MJ: Outbreak of bacteremic Campylobacter jejuni infection, 59(1):53–57
Sheehan HE: see Wedeen RP
Silver AL, Rose DN, editors: Urban community medicine: the Mount Sinai experience: honoring the work of Kurt W. Deuschle, 59(6):439–504
Sirota DK, Cobin RH, Futterweit W, Segal RL, Jacobs DR, Merker E: Hyperthyroidism in patients treated with lithium: report of nine cases, 59(1):79–82
Sivanandham M: see Wallack MK
Sledzinski Z: see Dobosz M
Social Science of Medicine: 59(6):461–468
Solomon G: see Rothenberg SP
Song HK: see Lehrer S
Speedling EJ: see Bloom SW
Sperling RS, Joyner M, Hassett J, Kee R, Wheatley P, Bauer C: HIV-1 seroprevalence in pregnant women testing positive on serologic screening for syphilis, 59(1):67–68
Spinal Cord Injuries: 59(2):163–169
Spirito N: see Petros JG
Spungen AM: see Bauman WA
Steinhagen RM: see Reissman P
Straus EW, editor: Festschrift for Rosalyn S. Yalow: hormones, metabolism, and society, 58(2):95–185; Introduction, Festschrift for Rosalyn S. Yalow, 59(2):95
Straus EW, Raufman JP: Brooklyn gastrinomas, 59(2):125–128
Student Corner: Familial adenomatous polyposis: case report and review of the extracolonic manifestations, KA Konsker, 59(1):85–91; Ileocecal tuberculosis in an Asian male immigrant: case report and literature review, MR Estill, 59(4):366–370
Student Prize, 1991: MR Estill, Ileocecal tuberculosis in an Asian male immigrant: case report and literature review, 59(3):191
Studies of the proposed mechanism regulating parietal cell emptying, G Nilsson, B Romell, K Girma, R Seensalu, 59(2):129–134
Successful successive studies in leukemia and breast cancer, JF Holland, 59(5):414–415
Sundaresan N: see Ralph Colp Prize
Surgery: 59(3):217–219
Surgical treatment of melanoma: what is controversial and what is not? M Shafir, 59(3):217–219
Synthetic antiproteases in acute pancreatitis: an experimen-

tal study, M Dobosz, Z Sledzinski, A Babicki, P Juszkiewicz, A Basinski, Z Wajda, 59(1):43–46
Syphilis: 59(1):67–68

Tachyarrhythmias, Ventricular: 59(4):318–325
Takemura Y, Ohnuma T: N^{10}-propargyl-5,8-dideazafolicacid (CB3717): Inhibitory effects on human leukemia cell lines resistant to methotrexate or trimetrexate, 59(5):419–424
Tamoxifen and endometrial cancer, RA Segna, PR Dottino, L Deligdisch, CJ Cohen, 59(5):416–418
Teratoma: 59(1):82–84
Thacker SB: see Koplan JP
Thromboxane: 59(2):157–162
Thung SN: see Lehrer S
Thymidylate Cycle: 59(5):391–395
Tormey MP: see Shandera WX
Treatment of a high-grade neurofibrosarcoma with concomitant vinblastine, doxorubicin, and radiotherapy, HW Bruckner, M Gorbaty, R Lipsztein, G Kranjac, MG Lebwohl, 59(5):428–431
Trimetrexate: 59(5):419–424
Tsurata M: see Bauman WA
Tuberculosis, Ileocecal: 59(4):366–370; Treatment and Prevention: 59(3):271–277

Urban Medical Centers: 59(6):479–486
Urban community medicine: the Mount Sinai experience: honoring the work of Kurt W. Deuschle, AL Silver, DN Rose, editors, 59(6):439–509; includes photos
Urinary Metabolites: 59(2):157–162

Vaccine, Hepatitis B: 59(6):487–492
Vaccinia Melanoma Oncolysate: 59(3):227–233
Vitale E: see Ramirez F

Wajda Z: see Dobosz M
Wallack MK, Scoggin SD, Sivanandham M: Active specific immunotherapy with vaccine melanoma oncolysate, 59(3): 227–233
Walsh JH: Physiology and pathophysiology of gastrin, 59(2): 117–124
Wang J: see Bauman WA
Waxman J: see Kahn H
Wedeen RP, Sheehan HE: Environmental hazards and public health: lessons for the practice of medicine, 59(1):23–27
Weinberger J: see Gaing AA
Weinstein IB: Protein kinase C: implications for cancer prevention and treatment, 59(5):387–390
Wheatley P: see Sperling RS
Why kidneys fail, LD Dworkin, 59(1):13–22
Wiernik PH: Advances in the treatment for Hodgkin's disease, 59(5):405–407
Winters SL: see Gomes JA

Yalow RS: Festschrift for Rosalyn S. Yalow: Hormones, metabolism, and society, EW Straus, editor, 58(2):95–185; illustrations: 59(2):96, 115, 124, 134, 185, inside back cover; Reminiscences and reflections, 59(2):97–101

Zhang RL: see Bauman WA
Zhong YG: see Bauman WA
Zollinger-Ellison Syndrome: 59(5):425–427

Kurt W. Deuschle in his garden, Dutchess County, New York, 1991.

THE
MOUNT SINAI JOURNAL OF MEDICINE

Forthcoming:

Grand Rounds

Narrative and Medicine
Oliver Sacks
Albert Einstein College of Medicine

Physician-Writers
Jack Peter Green
Mount Sinai School of Medicine

Osteoporosis: On the Verge of Rational Effective Therapy?
John Paul Bilezikian
Columbia University College of Physicians and Surgeons

The Global Impact of Penicillin: Then and Now
Gene H. Stollerman
Boston University School of Medicine

Obscure Gastrointestinal Bleeding
Blair Lewis
Mount Sinai School of Medicine

Pharmacology of Antiinflammatory Agents: A New Paradigm
Bruce N. Cronstein
New York University School of Medicine

Theme Issues

JANUARY 1993

Issues in Medical Ethics:
1. The Physician-Patient Contract
2. Transplantation
Editors: Rosamond Rhodes and Daniel A. Moros

MAY 1993

Quality Assurance
Editor: Suzanne Kramer

SEPTEMBER 1993

Cervical Disk Disease: Controversies in Neurosurgery
Editors: Kalmon Post and Martin H. Savitz

OCTOBER 1993

Issues in Medical Care for Childre
Editor: Richard Saphir

NOVEMBER 1993

Update on Sleep Disorders
Editor: Lee K. Brown
theme section in general issue

LEVY LIBRARY, MT. SINAI MEDICAL CENTER
3 4805 03008889 9

Available now:

Toward the Conquest of Pain
Allan P. Reed, editor
volume 58, no. 3, May 1991
84 pages **$15**

Festschrift for Rosalyn S. Yalow: Hormones, Metabolism, and Society
Eugene W. Straus, editor
volume 59, no. 2, March 1992
96 pages **$15**

Modern Management of Malignant Melanoma
Michail Shafir, editor
volume 59, no. 3, May 1992
86 pages **$15**

Subscriptions for 1992: $60 individuals, U.S. and Canada; $65 all libraries; $70 individuals outside U.S. and Canada.

To order subscriptions or copies of back issues ($15 each), please send check, payable to The Mount Sinai Journal of Medicine, to the Journal at Box 1094, One Gustave L. Levy Place, New York, NY 10029-6574; counter sales at Suite 1E, 50 E. 98th Street, New York, NY (phone: 212 241-6108; FAX: 212 722-6386).

MOUNT SINAI
GUSTAVE L.
AND
JANET W.
LEVY
LIBRARY

MOUNT SINAI
GUSTAVE L.
AND
JANET W.
LEVY
LIBRARY

LEVY LIBRARY, MT. SINAI MEDICAL CENTER
3 4805 0307482 6